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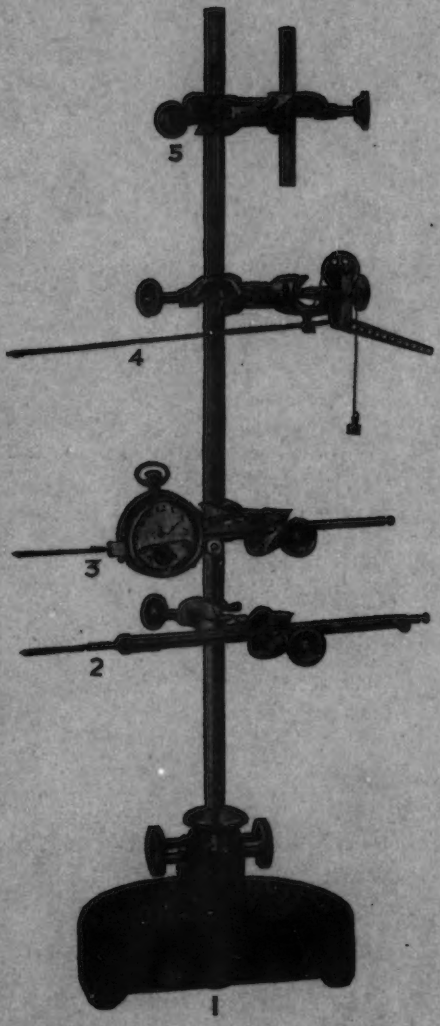

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FURTHER EVIDENCE ON THE PHYSIOLOGICAL MAXIMUM OF THE HEART

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In a previous publication (3) I made the statement that after bilateral vagotomy the heart beats at its physiological maximum rate, and that the injection of adrenalin or the stimulation of an afferent nerve failed to give any further increase in rate. As evidence for this statement I quoted an experiment in which the heart rate of that particular cat was 210 beats per minute after section of the vagi. This is a rather slow maximum heart rate, but nowhere in that publication was it asserted that this rate was the maximum rate for all cats. I have seen heart rates much higher than this, but since that experiment was typical of all other experiments along that line, it was quoted. Since it has been assumed by some readers that this was the maximum rate for any cat, I am not only giving additional tables with data to show that such is not the case, but I have brought forward fresh evidence upon the maximum heart rate which it is possible to get in the cat, under given conditions.

Cannon (1) has recently presented data of the heart rate in the cat with denervated heart which subsequently undergoes cerebral laceration. In his paper he makes the following statement: "Tulgan (THIS JOURNAL, 1924, lxix, 441) has declared that after bilateral vagotomy the heart rate is at its physiological maximum, and can not be increased by stimulation of a sensory nerve or by injection of adrenalin. In the figures he publishes the maximum rate for the cat is set down as 210 beats per minute. As is shown in figure 1 and in table 2 (Cannon's paper), this is not a physiological maximum in the sense that the heart is unable to beat faster than 210; it may beat very much faster than that. Furthermore, Cannon and Rapport (THIS JOURNAL, 1921, lviii, 330) have recorded the rate of approximately 260 beats per minute induced by the injection of adrenalin." He then asks the questions "Does the denervated heart differ from the heart still innervated by the sympathetics (as was the case in Tulgan's experi-

ments)? Does the nature of the anesthetic restrict the action of adrenalin?"

A part of the answer to the first question asked by Cannon is very simple since it is a well-known fact that the denervated heart has ordinarily a rate below the control rate. This may be demonstrated experimentally (see table below). Any increase in rate, therefore, which Cannon may get in the denervated heart, is not necessarily an increase above the physiological maximum, since the heart does not beat at its physiological maximum when completely denervated.

Cannon did not show that his observed rates were higher after complete denervation of the heart than when the accelerators were intact, nor does he give sufficient statistical evidence on which to base his argument.

In a former paper (4) some tables were presented showing heart rates when the stellates were removed following section of the vagi, and vice versa. These tables, fused into one for the sake of brevity, are reproduced in table 1.

TABLE 1

	HEART RATE
Control	202
After double vagotomy	218
After removal of the stellates	180
Control	173
After removal of the stellates	149
After double vagotomy	160

This serves to demonstrate that in dealing with the denervated heart we have to do with a set of conditions different from those under which the heart beats at its maximum. Conclusions, therefore, which are drawn from one set of conditions are not necessarily applicable to another set.

It has been further suggested that the anesthetic may be a factor influencing heart rate; in these experiments it was decided to eliminate the anesthetic in so far as possible by decerebration, to determine to what degree the anesthetic may act to interfere with the production of the maximum heart rate.

In a study of reciprocal reaction in the cardio-vascular system, the mechanism of response to changes in pressure by changes in heart rate under varying conditions of anesthesia, with vagi and stellates removed or intact was analyzed by Wickwire (5). I have followed this line of investigation in animals in which the cortex had been removed (1) anterior to the fissure of Rolando, and (2) when complete decerebration was done.

EXPERIMENTAL PROCEDURE. Ether anesthesia and tracheotomy were the routine in these experiments, but the anesthesia was intermitted

immediately after section anterior to the fissure of Rolando or complete decerebration. Usually not more than fifteen minutes intervened between the beginning of the experiment and the point where the anesthesia was stopped. Artificial respiration was resorted to in most of the experiments immediately after lesions of the cerebrum in order to wash out the ether as quickly as possible from the system. Preliminary to any brain operation, the skin was removed from the head and with forceps a small portion

Fig. 1

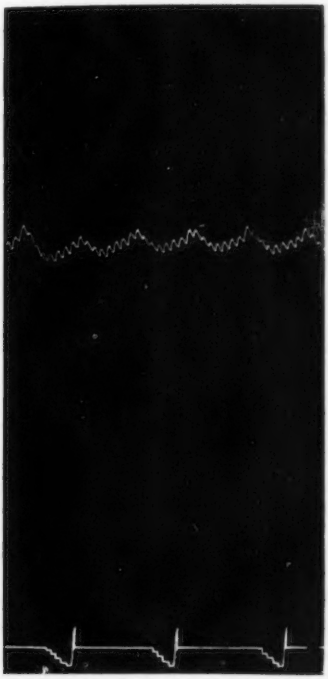

Fig. 2

Fig. 1. This figure shows the heart rate after double vagotomy.

Fig. 2. This shows the heart rate of the same animal but with the stellate ganglia removed subsequent to double vagotomy.

of the cranium was removed. Bone wax was used to stop hemorrhage, and as a result, most of these experiments were attended with practically no loss of blood. The blood pressure after this operation rarely fell more than 10 mm. and in many cases there was no fall at all. As soon as the animal was able to breathe on its own accord artificial respiration was dispensed with.

Three sets of experiments were performed, and protocols for each type of experiment are published.

1. Vagi divided followed by decerebration.
2. Vagi divided followed by
   a. Section of the cerebrum anterior to the fissure of Rolando.
   b. Compression of the abdominal aorta.
   c. Complete decerebration.
3. Section of the cerebrum anterior to the fissure of Rolando followed by
   a. Compression of the abdominal aorta.
   b. Complete decerebration.
   c. Section of the vagi.

EXPERIMENTAL RESULTS: *1. Vagi divided followed by decerebration.* After the vagi were divided the heart rate increased considerably, as did also the blood pressure. Subsequent removal of the cerebrum, with stopping of the anesthetic, failed to give any increase in heart rate above that observed after double vagotomy. Since there had been practically no loss of blood in the animal, and since the blood pressure was practically unchanged after decerebration, and since the complicating effect of the anesthetic had been removed, it would seem that there was no factor in the animal, after double vagotomy had been performed, which tended to keep the heart rate lower than the physiological maximum rate.

TABLE 2

	HEART RATE	
	June 4, 1925	June 5, 1925
Control rate under ether anesthesia	176	280
Vagi divided	240	292
Before decerebration with ether removed	240	292
After decerebration	196	284

Of course, one would expect, and one obtains, with very deep anesthesia certain changes in the reaction of the animal (Wickwire, loc. cit.), but with light anesthesia the changes are not very marked. In some unpublished data on hand now, I have observed a very slow rate of the heart, after double vagotomy, but upon removal of the anesthetic, and sufficient aeration of the tissues, the heart speeded up to a much higher rate.

The above experiment shows that light anesthesia does not markedly affect the heart rate, and that decerebration does not increase the rate above the rate observed after double vagotomy, but rather that it tends to lower the rate below that of the physiological maximum rate. Two protocols are given in table 2.

2. Vagi divided, followed by: a. Section of the cerebrum anterior to the fissure of Rolando. Upon section of the vagi, the usual sequence of events occurred—namely, an increase in the heart rate and a rise of blood pressure. Some very special heart rates were observed following double vagotomy.

On section of the cerebrum anterior to the fissure of Rolando, the heart rate becomes markedly slower, and since there is no longer the complicating effect of the anesthetic to take into consideration, the rate obtained may be considered as the true rate after section of the cerebrum anterior to the fissure of Rolando.

b. Effect of compression of the abdominal aorta. Wickwire (loc. cit.) has shown that compression of the abdominal aorta in an animal in which the vagi were divided with the accelerators intact, sometimes results in a compensatory response of a lower heart rate to the increased blood pressure, but the degree of compensation is much less than before the division of the nerves. In my experiments, besides division of the vagi, the cerebrum had been sectioned anterior to the fissure of Rolando. On compression of the abdominal aorta it was uniformly noticed that the response of a lower heart rate to the increased blood pressure was lost, the rule being an increase in heart rate to an increase in blood pressure. This, of course, is contrary to Marey's law. The heart rate, however, was never observed to be any greater, on compression of the abdominal aorta, than what it it was after double vagotomy had been performed. When the aorta is released, the usual compensatory response of an increase in heart rate to a falling pressure was uniformly observed. In this reaction Marey's law is followed, but the rate, again, never exceeded the rate observed after bilateral vagotomy.

TABLE 3

	HEART RATE	
	June 19, 1925	June 18, 1925
Control	172	240
During compression of the abdominal aorta	88*	232
After release of abdominal aorta	172	248
After double vagotomy	252	280
During compression of the abdominal aorta	244	260
After release of the abdominal aorta	252	260
After section of the cerebrum anterior to the fissure of Rolando	240	252
Compression of the abdominal aorta	252	264
After release of the abdominal aorta	252	268
After complete decerebration	232	260
Compression of the abdominal aorta	224	224
After release of the abdominal aorta	224	224
At the end of the experiment	172	180

* See figure 3. This rate was so unusually low on compression of the abdominal aorta, that the tracing is published.

c. Complete decerebration. After the cerebrum had been completely removed the change in heart rate was not very great. Usually the rate

went up slightly above what it had been after section anterior to the fissure of Rolando, although in some instances it decreased somewhat. The changes, however, were not large in either direction and the rate never exceeded in any case after complete decerebration the rate present after bilateral vagotomy.

During compression of the abdominal aorta after decerebration, it was observed that Marey's law of inverse ratio of heart rate and blood pressure applies.

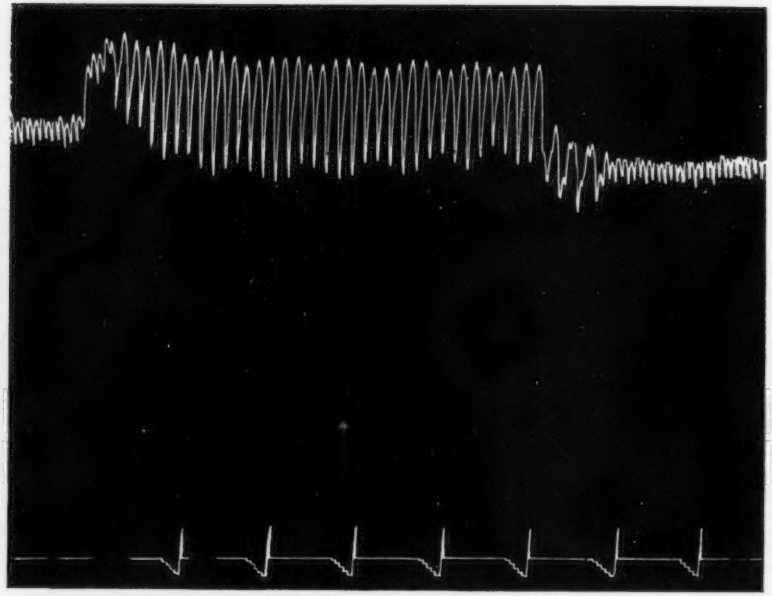

Fig. 3. The effect of compression of the abdominal aorta on heart rate, with the vagi and accelerators intact. This rate, which is 88 beats per minute, is so unusually slow, and the amplitude so great that this tracing is published.

A protocol of all the above mentioned procedures is given in table 3.

3. Section of the cerebrum anterior to the fissure of Rolando followed by: a. Compression of the abdominal aorta. In this series of experiments the cerebrum had been sectioned anterior to the fissure of Rolando without any lesions to the cardiac nerves. It was desired, here, to do all the experiments done above with the vagi intact followed by double vagotomy after all other procedures had been done, and the heart rates noted. After section anterior to the fissure of Rolando no anesthetic was employed, hence avoiding this factor in the equation. If there is an increase in heart

rate when the vagi are sectioned subsequent to all previous lesions, such as section of the cerebrum anterior to the fissure of Rolando and complete decerebration, one is justified in concluding that under these conditions, the maximum rate of the heart is reached only after sectioning of the vagi. Upon section of the cerebrum anterior to the fissure of Rolando, the usual slowing of the heart rate as observed above, is again noticed.

On compression of the abdominal aorta after section of the cerebrum anterior to the fissure of Rolando, the heart behaved in all ways as if there had been no lesion of the brain. There was a compensatory response of heart rate to falling pressure, and Marey's law applies. This is just contrary to what happened when the abdominal aorta was occluded, subsequent to bilateral vagotomy and section of the cerebrum anterior to the

TABLE 4

	HEART RATE	
	June 23, 1925	June 25, 1925
Control	208	240
During compression of the abdominal aorta	108	284* 140
After release	228	196* 200
After section anterior to the fissure of Rolando	192	224
Compression of the abdominal aorta	172	204
After release	200	244
After complete decerebration	188	208
Compression of the abdominal aorta	176	204
After release	188	244
Vagi sectioned	216	284

* Very deep anesthesia (see Wickwire, loc. cit., "reversed reaction"). The figures 140 and 200 were given by the same animal in a later compression when the anesthesia was light.

fissure of Rolando, as will be noticed on consulting table 2. Upon releasing the pressure the heart increases in response to the falling pressure.

b. Complete decerebration. After complete decerebration there was in every case a uniform decrease in heart rate. Marey's law, however, held when the abdominal aorta was compressed and also upon the release of the pressure.

c. Section of the vagi. This portion of the experiment was considered the most crucial for if the heart rate now increased after bilateral vagotomy it would prove that in all the experiments in this series up to this point the heart could not be made to beat at its physiological maximum by any procedure or even the absence of the anesthetic. Since, as the table above shows, a decided increase in heart rate was obtained after bilateral vagotomy, we are safe in concluding that the physiological maximum rate of the heart is obtained only after bilateral vagotomy, and that light

anesthesia does not interfere to any marked degree with the maximum heart rate.

It will be noticed in table 4 that the maximum rate of the heart was not obtained until after double vagotomy had been done, although the anesthetic had been removed long before this operation.

Discussion of results. It must be clearly understood, since there are sufficient experimental data on hand, that the heart of an animal which is completely denervated, reacts to nervous or chemical changes in the body of that animal in a different manner from one in which the vagi have been sectioned leaving the sympathetics intact. Hooker (2) first

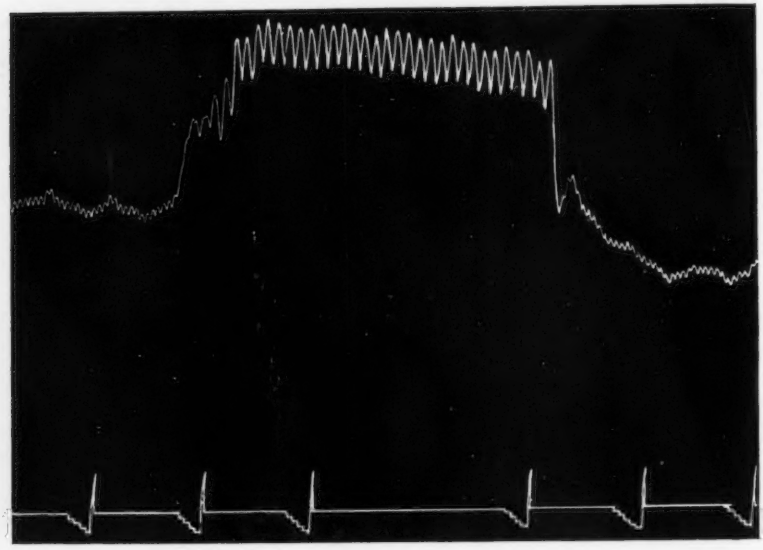

Fig. 4. Effect of compression of the abdominal aorta after double vagotomy. The rate is unusually slow for an animal with both vagi sectioned.

advanced the theory that there is a physiological maximum rate of the heart which is usually reached after section of the vagi, and beyond which it can only with difficulty be increased. I have, in a previous paper, confirmed Hooker's results. Recently Cannon (loc. cit.) working with animals in which the heart had been denervated, and which were later decorticated, maintained that figures which I had previously published, did not represent the physiological maximum rate of the heart. This statement is not supported either by a comparison of the observed rates with the rate when the accelerators were intact, or by sufficient statistical data to show that the observed rate was well beyond the range of the heart when the accelerators were intact.

Cannon's experiments, too, show that in the case of the denervated heart, when the animal is in a pseudaffective state, and it manifests a variety of movements, there are changes in heart rate. But the fact must be borne in mind that the denervated heart beats at a lower rate than the heart which is innervated by the sympathetics alone, hence any increase which is obtained under any of these conditions does not necessarily mean an increase above the physiological maximum which one might expect to obtain from these animals. The rates observed after bilateral vagotomy but with the accelerators intact may be higher than those observed by Cannon in the denervated heart. There is, then, no evidence at present tending to show that the deportment of the denervated heart varies in essential particular from the law of the physiological maximum rate. It is interesting to note in this connection that in decerebrated animals, in which the vagi are cut, but with the accelerators intact, no increase above the rate present after double vagotomy was ever obtained. The law of the physiological maximum rate of the heart evidently seems to hold in such animals.

That light anesthesia, sufficient to abolish pain in the animals experimented on, does not interfere to any marked degree with increases in heart rate, is also established by these experiments, for if the vagi are sectioned subsequent to decerebration, with removal of the anesthetic, there is a marked increase in the heart rate and that rate is the highest rate observed, no matter what the experimental lesion was preliminary to double vagotomy. It is also a well-established fact that the stimulation of the sciatic nerve in an etherized animal in most cases results in an increase in the heart rate. Of course with very deep anesthesia we may get different results, but in most experimental work, the animal is anesthetized sufficiently to obtain surgical anesthesia in which condition all sense of pain is lost. This, however, does not mean that the degree of anesthesia is so deep as seriously to interfere with the conclusions so obtained.

CONCLUSIONS

1. The denervated heart can not be considered to respond to certain changes in the animal, in the same way as the heart which is still innervated by the sympathetics but with the vagi sectioned.

2. Further evidence is presented to show that the physiological maximum rate of the heart is obtained only after bilateral vagotomy.

3. There is no evidence at present that the denervated heart departs from the law of the physiological maximum rate.

4. Light anesthesia, sufficient to produce surgical anesthesia, does not seem to interfere to any marked degree with the rate of the heart after double vagotomy and does not interfere with the physiological maximum rate thus obtained.

5. There is a reversal of Marey's law of inverse ratio of rate to pressure when the abdominal aorta is compressed subsequent to section of the cerebrum anterior to the fissure of Rolando.

6. With complete decerebration, Marey's law holds.

I wish to express my sincere thanks to Dr. Helen C. Coombs for many suggestions, and valuable criticisms, and also to Prof. F. H. Pike for reading and criticizing the manuscript.

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BIOLOGICAL FOOD TESTS

IX. Vitamin A in Three Varieties of Cheese

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It is obvious that cheese made from whole or half-cream milk is apt to retain a large part of the vitamin A content of the milk fat. This assumption has been incorporated in most of the current teaching, although published evidence is lacking. The statement by Sherman and Smith (1922) that ripened cheese has a high vitamin A value is based upon unpublished work by Sherman.

Several factors may govern the completeness of retention of the vitamin A content of the milk from which it is made in the ripened cheese. The vitamin potency of the milk used, the per cent of milk fat in the final product, the destruction of vitamin by the heating and aging involved in cheese making, and the possible effect of bacterial agencies may all play a part in this process. It is plain that all types of cheese cannot be expected to be of equal value as sources of vitamin A since all these factors may vary not only among types but even among samples of the same type. It is to be expected that cheese which contains a high per cent of milk fat, 50 per cent of dry matter or more, will provide more vitamin A than that which contains a smaller amount of fat. Whether the temperature used in curd coagulation and the length of ripening period as well as the strain of bacteria involved have also an effect upon this characteristic of the cheese are questions which can be answered only by experiment.

Standards of cheese selected. Three types of cheese, American Cheddar, Limburger and Swiss (Emmenthaler) were chosen for the study here reported. The official definitions of these types of cheese published by the United States Department of Agriculture in 1921 in Food Inspection Decision 181 (U. S. Dept. Agric. 1921) as a guide for officials in enforcing the Federal Food and Drugs Act are as follows:

Cheddar cheese, American cheese, American Cheddar cheese, is the cheese made by the Cheddar process, from heated and pressed curd obtained by the action of rennet on whole milk. It contains not more than 39 per cent of water, and, in the water-free substance, not less than 50 per cent of milk fat.

Limburger cheese is the cheese made by the Limburger process, from impressed curd obtained by the action of rennet on whole milk. The curd is ripened in a damp

atmosphere by special fermentation. It contains in the water-free substance not less than 50 per cent of milk fat.

Emmenthaler cheese, Swiss cheese, is the cheese made by the Emmenthaler process from heated and pressed curd obtained by the action of rennet on whole milk, or on partly skimmed milk, and is ripened by special gas-producing bacteria, causing characteristic "eyes" or holes. The cheese is also known in the United States as "Schweitzer." It contains in the water-free substances, not less than 45 per cent of milk fat.

Analyses of these varieties of cheese as given in the United States Department of Agriculture Bulletin 28 (Atwater and Bryant, 1903) are as follows:

	NUMBER OF ANALYSES	WATER	PROTEIN	FAT	CARBOHYDRATE	ASH	FAT ON WATER-FREE BASIS
Cheese, full cream (cheddar).....	25	34.2	25.9	33.7	2.4	3.8	51.2
Cheese, limburger.....	1	42.1	23.0	29.4	0.4	5.1	50.8
Cheese, Swiss (Emmenthaler).....	2	31.4	27.6	34.9	1.3	4.8	50.0

Methods of manufacture. According to Doane and Lawson (1918), considerable differences exist among methods used in the production of these three types of cheese. The *Limburger*, which is a soft cheese, is set with rennet at 91° to 96°F., the curd cut and set away for ripening without pressing, and with no further heating. A good deal of salt is used in curing this cheese during the ripening period of one to two months.

The *Cheddar* and *Emmenthaler* are hard cheeses, both of which are set in much the same way as the Limburger. The *Cheddar* cheese is heated slowly with stirring to 96° to 108°F. and kept at about 90°F. for one to three hours, while the curd is being cut, matted and drained. After being pressed, usually over night, the cheese is removed to the curing room for a ripening period of three to twelve days, after which it is placed in cold storage. Formerly the ripening period extended to six months.

In the manufacture of *Emmenthaler* or *Swiss* cheese as practiced in Switzerland, after coagulation of the milk the curd is cut very fine, then heated to 126° to 140°F. for about one hour with stirring until the curd becomes firm. It is then drained, salted and ripened at 55° to 65°F. from six to ten months or longer. This period is reduced about one-half in the United States.

Apparently the process used in the manufacture of Swiss cheese involves exposure of the milk fat to longer periods of heating and stirring at higher temperatures than is the case with Cheddar and Limburger cheeses.

Methods of vitamin testing. All rats used in these tests had been depleted of vitamin A reserves by a fore-period of somewhat varying length. During the whole experiment the animals were fed the usual basal diet previously described (Morgan, 1923). Dry brewery yeast was fed as source of

vitamin B in 0.5 gram daily doses separately from the rest of the diet. All animals were kept in separate cages equipped with wire screen false bottoms.

Antirachitic check. Since full growth was obtained in the cheese-fed rats even in that group which continued to show ophthalmias throughout the experiment it was assumed that sufficient antirachitic material was furnished by the cheese and therefore no irradiation was carried out. The need for caution on this point in all vitamin A testing as suggested by Steenbock, Nelson and Black (1924), is recognized however and the practice of irradiation in connection with such work is now established in this laboratory.

The cheese samples used were from the same lot throughout and were kept in the ice chest. No chemical analyses of these samples were attempted.

Results of Cheddar cheese tests. Five rats which showed by falling weight and ophthalmias a severe condition of vitamin A deficiency were each given at first for a period of two weeks one gram daily of California full cream cheese, Cheddar type. This was then reduced to one-half gram daily. Unfortunately, just at this time for a period of about four weeks an inferior lot of yeast was received with consequent delay in the growth of these animals. The eye condition, however, which was particularly severe in 1004 and 771 cleared up within a day or two after the first administration of the cheese. By comparison with the growth of rat 1003, which was of the same lot but which was given one-half gram of butter-fat daily instead of the cheese, it is evident that the cheese-fed rats made normal progress.

All of the rats when mature were mated but no young were born of the females. Both males, however, when mated with other females were shown to be fertile by the production of litters. Nearly 50 per cent fertility has been obtained in this laboratory in some fifty matings of females reared from weaning on our basal diet which contains 20 per cent of Crisco. The direct influence of the cheese upon fertility is therefore hardly demonstrable by the few cases here reported.

These results are illustrated in table 1.

Results of Limburger cheese tests. Four rats showing unmistakable evidence of vitamin A deficiency were given at first one gram daily of Limburger cheese, then after two weeks, one-half gram daily. The ophthalmias in these animals were almost immediately relieved, and their growth even on the half-gram dosage was entirely normal, as shown in table 1. One of the three females mated produced a litter of nine normal young, only one of which she weaned.

Results of Swiss cheese tests. Four rats showing severe vitamin A deficiency were placed at first upon one-half gram daily dosage of Emmenthaler cheese imported from Switzerland. After three to four weeks when growth

and eye condition were found to remain unsatisfactory, the dosage was raised to one gram daily. Upon this amount of Swiss cheese the animals showed normal growth, but in three of the four cases never cleared up the eye trouble entirely. The fourth animal 1001, a female, developed satis-

TABLE 1

Effect of three types of cheese upon vitamin A deficiency in rats

NUMBER OF RAT	SEX	TYPE OF CHEESE	AMOUNTS FED	AVERAGE GAINS IN 12 WEEKS ON CHEESE	CONDITION OF ANIMALS AFTER 20 WEEKS
				grams	
756	♀	American full cream Cheddar type (from California)	1 gram daily for 2 weeks, then 0.5 gram daily	36	Excellent nutritive condition, no eye disease, normal size. No young from 3 females mated, but the 2 males were proved fertile
771	♀			74	
1010	♀			59	
770	♂			94	
1004	♂			101	
981	♀	Limburger, full cream (from New York)	1 gram daily for 2 weeks, then 0.5 gram daily	100	Excellent condition, no eye disease, growth rate normal. One out of 3 females mated produced 9 young, weaned one
983	♀			93	
995	♀			71	
986	♂			122	
1001	♀	Swiss, full cream, Emmenthaler type (from Switzerland)	0.5 gram daily at first, raised to 1 gram after 3 to 4 weeks	79	All 4 animals showed persistent ophthalmias, but growth rate was normal. The one female mated produced 2 young; both died
982	♂			106	
993	♂			87	
998	♂			120	
				(On butter)	
1000	♀	Butter-fat (controls)	0.5 gram daily	76	All made normal growth and rapid recovery from ophthalmias. The female produced 4 young, all of which died
1003	♂			109	
1009	♂			81	

factorily and produced a litter of two young, both of which died in a day or two.

Discussion. There would seem to be a definitely smaller content of vitamin A in imported Swiss cheese than in American Cheddar and Limburger cheese. Although the Swiss cheese tested was sold as a whole

milk product the legal standard for this type of food allows a somewhat lower per cent of milk fat than that required of the other two kinds. The difference, that between 45 and 50 per cent of the water-free solids, is hardly sufficient, however, to explain the decrease in vitamin A here reported, for even with twice the amount of cheese given the other rats those on Swiss cheese showed the persistence of eye infection characteristic of low vitamin A levels. The slight difference in procedure of testing between the Cheddar and Limburger on the one hand and the Swiss on the other can hardly account for the persistent difference in ophthalmia conditions.

Whether the higher temperatures involved in the Swiss cheese-making process, the particular variety of bacterial action characteristic of this cheese, or the longer period of ripening is most at fault in producing the vitamin destruction cannot be stated. All three of these factors may be involved. At any rate, the full protective action of the approximately one-third gram of butter-fat in the Swiss cheese dosage was not present.

The Cheddar and Limburger cheese compared well with butter-fat in these tests, as good results being obtained with one-half gram of these cheeses as have been obtained in this laboratory upon a minimum of one-fourth gram of butter-fat (Morgan, 1923a). Since less than one-fourth gram, probably about one-sixth gram of butter-fat, was present in the cheese doses fed, an advantageous concentration of the total vitamin A of the milk appears to result from these types of cheese curding. Similar studies upon other types of cheese are under way in this laboratory.

SUMMARY

1. Young rats suffering from vitamin A deficiency recovered rapidly from the usual eye disease and made normal growth upon addition to their diet of one-half gram daily portions of California cream cheese (Cheddar type), or of Limburger cheese (from New York). These cheeses appear to retain in an unusually concentrated form the vitamin A of the milk from which they are made.

2. Under similar circumstances one-half gram doses of Swiss cheese (from Switzerland) did not cure ophthalmias nor restore growth. With one gram doses growth was normal but eye disease persisted to some extent. The deficiency of this cheese may be due either to the relatively long heating and curing processes used or to selective bacterial action.

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THE EFFECT OF INSULIN ON THE RESPIRATORY EXCHANGE OF DECEREBRATE AND DECAPITATE CATS

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Dickson, Eadie, Macleod and Pember (1924), working with dogs, have shown that after injection of insulin there occurs a rise in respiratory quotient, then an increase in respiratory volume and oxygen consumption, and finally accelerated respiration and pulse. These changes follow the initial fall in blood sugar and are often associated with hyperexcitability. In rabbits they were unable to demonstrate an increase in respiratory volume, there was only a doubtful increase in oxygen consumption, but there was a definite rise in the respiratory quotient of three out of four preparations, though it fell later. Dudley and co-workers (1923) found in mice a fall in the oxygen consumption and respiratory quotient. Burn and Dale (1924) published results on decapitated and eviscerated cats, showing a rise in oxygen consumption following intravenous injection of insulin. The respiratory quotient, even in their control preparations, approached unity, which they suggest is characteristic of the metabolism of muscle tissue. Krogh and Brandt-Rehberg (1925) found that in urethanized and curarized rabbits the respiratory quotient rises shortly after injection of insulin and reaches a maximum in 1 to 3 hours. This maximum was never as high as 1 and only rarely went to 0.95. Their conclusion is that insulin changes the type of metabolism from that ordinarily seen in resting animals to one in which carbohydrate is burned.

1. Experiments with decerebrate cats. The cats were prepared in two ways: *a*, with the Sherrington guillotine, and *b*, by the method of Pollock and Davis (1923), i.e., tying off the carotid arteries in the neck and the basilar artery through a hole in the base of the skull. The expired air was collected for analysis in a rubber bag after having passed through a sensitive meter which recorded the volume. The valves employed were those described by Macleod (1922). Samples of air drawn at intervals of ½ to 1 hour were analyzed in a modified Haldane gas-analysis apparatus. Small quantities of blood were taken directly from a carotid artery, and blood sugar determinations made by the Hagedorn-Jensen method, and later by a modified Shaffer-Hartmann technique in which 0.2 cc. quantities of blood were used.

In 9 preparations the ventilation, oxygen consumption and respiratory quotient were determined with very contradictory results. Difficulty was experienced due to the fact that the great majority of these preparations develop rapid, gasping respirations (see Bazett, 1924, and Olmsted and Taylor, 1926). In only one, cat 33 (fig. 1) was a complete analysis obtained without any symptoms of respiratory failure complicating the results. In this preparation the ventilation, oxygen consumption, CO_2 output, and respiratory quotient remained at a constant level for an hour

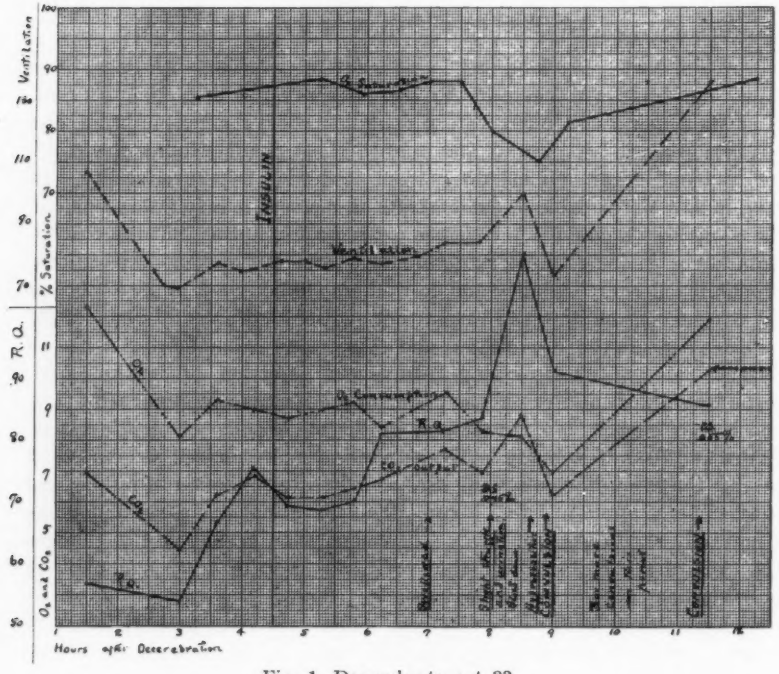

Fig. 1. Decerebrate cat 33

and a quarter following injection of insulin. The first to show a change was the respiratory quotient which rose from its former level of 0.70 to 0.81. Two hours after insulin the CO_2 output and the rate of ventilation first showed a tendency to rise, and two hours later had reached their maximum. This proved to be the period just preceding convulsions. The oxygen consumption remained level for three hours, then it fell from 0.9 to 0.7 litre per hour over a period of $1\frac{1}{2}$ hours, at the end of which time there appeared premonitory signs of an approaching convulsion. Preceding the convulsion, therefore, and when hypersensitivity was marked, it was noted

that the ventilation and CO_2 output had increased greatly and the respiratory quotient had risen to 1.1, while the oxygen consumption had decreased. Immediately following the first seizure, the ventilation, CO_2 output and respiratory quotient fell, and the oxygen consumption still remained low. During the next two hours the animal passed through 5 or 6 violent convulsions at the end of which the ventilation, CO_2 output, and oxygen consumption all had increased greatly, and the respiratory quotient had fallen to 0.85.

In the other 8 preparations, in spite of difficulties with the respiration, there was in every case a definite rise in respiratory quotient 3 to 4 hours after injection of insulin.

2. Experiments with decapitate cats. The difficulties in the way of obtaining and interpreting observations on decerebrate cats, and the suggestive observations on the oxygen consumption of cat 33, led us to use decapitate animals, thus ruling out the possibility of direct stimulation of the respiratory center being a complicating factor in the interpretation of respiratory analyses. By eliminating muscle spasm with curare in some cases, we simplified the conditions still further.

The cats were decapitated in the usual way. For the ventilation of the preparation we devised a mechanical valve to attach to an air pump, which would insure constant ventilation, and at the same time allow us to collect the expired air for analysis.

The valve consisted of a hollow brass cylinder in which rotated an accurately fitting solid cylindrical plug. There were four holes in the hollow cylinder communicating by means of short tubes with the pump, the tracheal cannula of the cat, the air sampling bag and the outside air. In the cylindrical plug there were two grooves, one much longer and deeper than the other. During compression of the piston in the pump, the larger groove established connection between the tube from the pump and that to the tracheal cannula, while the tubes leading to the sampling bag and the air intake were closed off. Just at the end of the piston stoke the plug was turned by a moving shaft so that the large groove now connected the tracheal tube to the sampling bag and the air from the inflated lungs of the preparation passed without resistance to the sampling bag. At the same time the tube to the pump became connected through the small groove with the intake opening, and as the piston of the pump was pulled out air was drawn into the pump which now had no connection with the cat. That this arrangement did supply a practically constant ventilation was shown by a number of experiments in which the air expired from the cats was measured from time to time in a meter. In one experiment 32 readings over a period of 10 hours gave a maximum deviation of 2 per cent from the average. In another there was a maximum deviation of 1.8 per cent over a period of 9 hours.

Control preparations. Three decapitate cats were observed as control preparations. All three showed a fairly constant respiratory exchange throughout the experiment. Cat 93 (fig. 2) was followed for $15\frac{1}{2}$ hours,

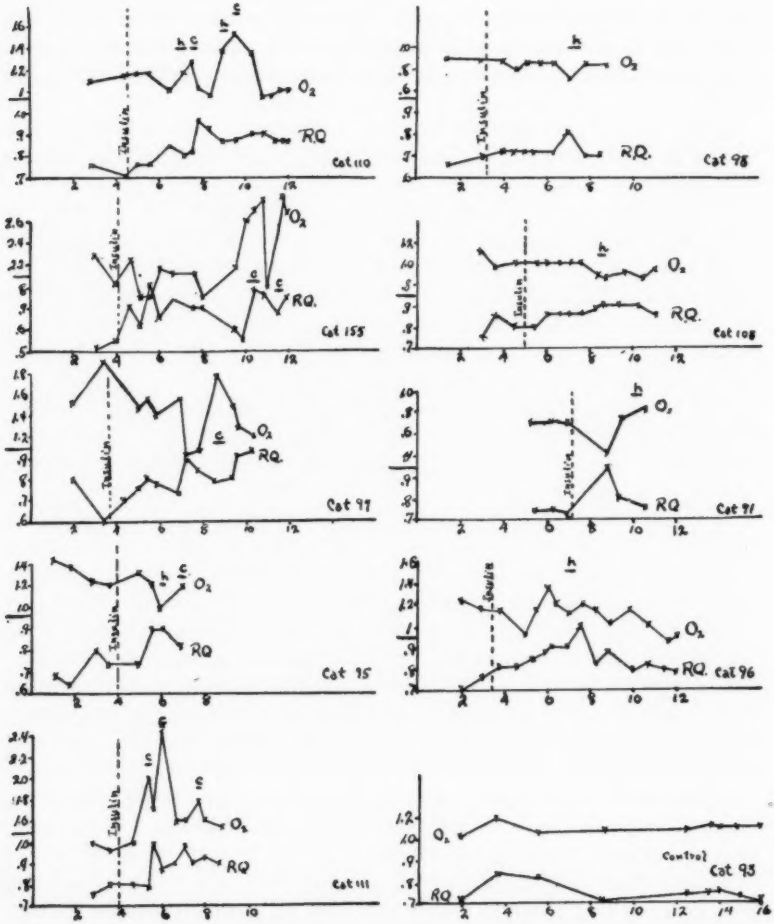

Fig. 2. Decapitate cats. Abscissae = hours after decapitation. Upper curve = O_2 consumption in litres per hour. Lower curve = *R.Q.* H. = hypersensitive. *C* = convulsions.

and with the exception of an initial variation, maintained a uniform rate of O_2 consumption and CO_2 output. The average respiratory quotient was 0.72 for the three preparations.

Preparations injected with insulin. Nine preparations were injected with insulin. Of these, five developed typical, violent convulsions (Olmsted and Taylor, 1926), while four showed only hyperexcitability with occasional stretching or scratching. The results obtained from these nine cats are shown in figure 2.

A. Oxygen consumption. The most marked increase appears to be directly related to the convulsions. In four of the five cats in which typical convulsions appeared (fig. 2, cats 110, 155, 97, 95 and 111) there was no striking increase in the O_2 used until convulsions appeared, and then it increased greatly. Following convulsions it returned to its former level. In the fifth cat (cat 111), convulsions appeared 1 hour after insulin and the air sample taken at this time showed a marked increase in O_2 consumption and there was no return to the original level. Of the four preparations which did not develop convulsions, two (cats 98 and 108) showed no rise in O_2 consumption, the other two (cats 91 and 96) showed a slight rise when hypersensitivity and scratching movements were marked.

A marked fall in the O_2 consumption was seen in cat 97 during the period of hypersensitivity preceding convulsions. The O_2 consumption fell from a level of 1.45 to 1.10, remained at this point for half an hour, then rose to 1.68 during convulsions. A similar though less striking fall during the period of hyperexcitability is seen in cat 95. In short, in eight out of the nine preparations there are indications of a fall in O_2 consumption just before or during the period of hypersensitivity which precedes convulsions. In the exceptional case (cat 111) convulsions appeared so early after insulin, within an hour, that the fall in O_2 consumption either escaped detection or did not occur.

B. The respiratory quotient. The respiratory quotient rose in all nine preparations. This is shown in table 1. The first five preparations in the table showed a definite rise in the level of the respiratory quotient, with increases over this higher level during the period of hyperexcitability, coinciding with the lowered O_2 consumption. In the other four there was a more gradual rise to the maximum which again coincided with the period of hyperexcitability in three out of the four preparations. Later there occurred a fall in the respiratory quotient.

There appears to be a very definite relation between the extent and duration of the rise in respiratory quotient and the severity of the symptoms following insulin. The respiratory quotient in those preparations in which severe and prolonged convulsions were noted tended to show a rapid rise to a high value which was maintained for a considerable time. In those which did not develop convulsions, the rise was less rapid, less in extent, and was not maintained.

A typical result is seen in cat 110. Preceding insulin and for 1½ hours following it, the O_2 consumption, CO_2 output and respiratory quotient

remained steady. About 2 hours after insulin the CO_2 showed a slow, steady rise, but the O_2 consumption dropped, and the respiratory quotient correspondingly rose. Within a few minutes of taking of the sample a convulsive struggle was noted. This was followed by twitching and stretching, over a period lasting nearly an hour, ending in a series of violent convulsions. During the period leading up to the convulsions the O_2 consumption had risen and the respiratory quotient fallen, but not to its original level. During the convulsions both the O_2 consumption and the CO_2 output rose sharply, but the respiratory quotient remained steady. Following the convulsions, the cat was very quiet, knee jerks were absent, and it did not appear in the least hypersensitive. An hour elapsed before further symptoms appeared and during this time the O_2 consumption and

TABLE 1

Respiratory quotient in decapitate cats injected with insulin

CAT NUMBER	STARVED OR FED	RESPIRATORY QUOTIENT			LOWEST BLOOD SUGAR	SYMPTOMS
		Average level before insulin	Average level after insulin	Highest point reached		
111	Starved	0.78	0.92	1.01	0.034	Convulsions
110	Starved	0.75	0.87	0.95	0.056	Convulsions
155	Starved	0.56	0.70	0.80	0.023	Convulsions
97	Starved	0.70	0.80	0.90	0.054	Convulsions
95	Starved	0.70	0.86	0.89	0.046	Convulsions
96	Starved	0.80	—	0.98	0.059	Hyperexcitable and scratching
91	Not starved	0.75	—	0.95	0.060	Hyperexcitable and scratching
108	Not starved	0.80	—	0.91	0.045	Hyperexcitable with a little scratching
98	Starved (?)	0.70	—	0.82	0.068	Hyperexcitable only

CO_2 output fell rapidly, the former to a greater extent than the latter, so that the respiratory quotient again rose, this time to 0.95. Stretching, hypersensitivity and convulsions again supervened with a marked rise in O_2 consumption and CO_2 output which reached a maximum toward the end of the convulsive period, and was associated with a decrease in respiratory quotient. Following the period of convulsions, the O_2 consumption and CO_2 output fell rapidly to slightly below the original level in the case of the O_2 and to the level at the beginning of convulsions in the case of the CO_2. The respiratory quotient showed a slight rise. The cat showed no more symptoms, and the O_2 consumption and respiratory quotient remained fairly steady for 1½ hours, when the cat was killed. At this time it was in perfect condition. Reflexes were present, the heart's action

good and on cutting off the air supply, it developed violent asphyxial convulsions.

These findings show that the insulin causes a definite rise in the respiratory quotient in the decapitate cat, but probably does not cause any very

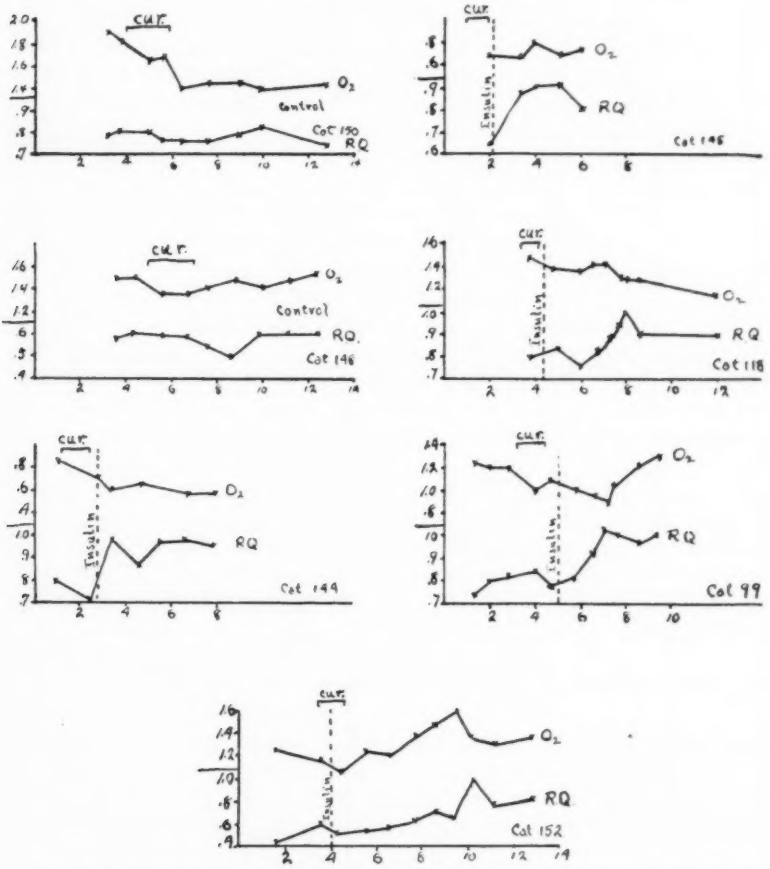

Fig. 3. Curarized decapitate cats. Abscissae = hours after decapitation. Upper curve = O_2 consumption in litres per hour. Lower curve = *R.Q.*

marked rise in the O_2 consumption, the greatest increase in O_2 consumption being due to the muscular spasms. On the contrary, preceding the convulsions there may be a small but significant decrease in the O_2 consumption, coincident with the rise in respiratory quotient.

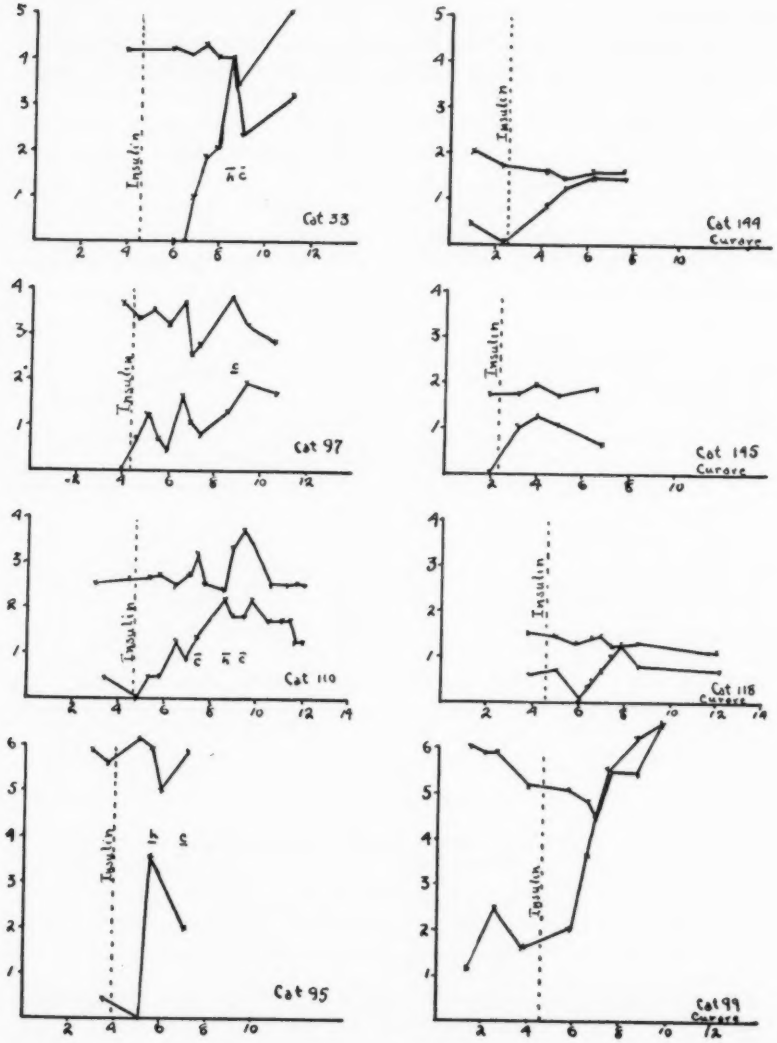

Fig. 4. Caloric output. Abscissae = hours after decapitation. Upper curve = total caloric output. Lower curve = calories due to combustion of carbohydrate. *H* = hypersensitive. *C* = convulsions. Cat 33, decerebrated, the others decapitated. Cats 144, 145, 118 and 99 curarized.

3. Curarized decapitate cats. Curare was administered to ten decapitate preparations, four serving as controls, and six being given insulin. The controls showed little variation in respiratory exchange throughout the experiment (fig. 3, cat 148) but in three there was a general lowering of the whole metabolic rate as indicated by the lowered O_2 consumption as in cat 150. The effect of curare on the blood sugar is of some interest. In the control preparations the blood sugar curare showed the characteristic fall from above 0.2 per cent to 0.1 per cent within six hours. The blood sugar of those curarized preparations which had been given insulin was in cat 144, 0.02 per cent 4 hours after insulin; in cat 145, 0.037 per cent in $3\frac{3}{4}$ hours; in cat 118, 0.036 per cent in 4 hours; in cat 49, the lowest point reached was 0.067 per cent in $1\frac{1}{4}$ hours; in cat 152, 0.062 in $1\frac{3}{4}$ hours. None of the usual symptoms appeared in spite of the low blood sugar, and the animals remained perfectly quiet and motionless until the effect of the curare wore off several hours later. Observations made on preparations which showed any movement in response to severe pinching were discarded. The pulse was followed as an indication of the condition of the preparation.

A. The oxygen consumption remained fairly steady in two preparations (fig. 3, cats 144, 145); there was a temporary slight rise 2 hours after insulin followed by a fall in another (cat 116); in another, a decided fall followed by a rise (cat 99); in the last a marked rise interrupted by a slight fall (cat 152).

The variations in O_2 consumption in three of the five preparations were of the same magnitude as in the controls. In two, however (cats 99 and 152), there was a considerable rise in O_2 consumption four or five hours after insulin. This is about the time when the uncurarized preparations are due to have convulsions, and when the blood sugar of many of the curarized preparations is below the convulsions level, but it was in these two the blood sugar did not fall below 0.060 per cent.

B. The respiratory quotient was definitely raised in every case, and the value reached was nearly unity.

From our data it is possible to calculate by using Lusk's tables the total caloric output of our preparations at different periods after injection of insulin, and also to calculate the calories consumed as carbohydrate and those consumed as fat. The results of the calculations on eight preparations are given in figure 4.

In the one successful decerebrate preparation (cat 33), the total caloric output remained fairly level until convulsions appeared. On the other hand the caloric output due to carbohydrate combustion rose rapidly from zero to a maximum at the time when hypersensitivity was noted preceding convulsions, and at this period the total caloric output was due to carbohydrate combustion. At this time also the blood sugar was at

its lowest point, 0.045 per cent; likewise the oxygen saturation of hemoglobin and oxygen consumption were both low, in spite of increased ventilation; and the respiratory quotient was 1.1. After convulsions there was a rise in the total metabolism, in the blood sugar, in the O_2 saturation of hemoglobin, in the O_2 consumption and in the ventilation, whereas the respiratory quotient and caloric output due to carbohydrate consumption showed a decline.

In the decapitate cats there was in general the same effect. The total caloric output varied slightly from hour to hour, but on the whole the general level remained the same. There was usually, however, a distinct fall before convulsions appeared. The caloric output due to carbohydrate combustion on the other hand, rose from zero or a low level, until in five out of the eight examples it accounted for all the energy output just before the time for convulsions. In other words the metabolism became purely of the carbohydrate type. This is the point made by Krogh and Brandt-Rehberg (1924).

SUMMARY

In a decerebrate cat injected with insulin in which there were no complications through respiratory distress, the respiratory quotient rose, but the O_2 consumption remained level until just before convulsions, when it fell. After a series of convulsions the respiratory quotient fell, while the O_2 consumption and ventilation rose.

In decapitate cats injected with insulin the O_2 consumption generally falls temporarily either just before or at the time when hypersensitivity is observed. A rise in O_2 consumption is usually associated with muscle spasms. The respiratory quotient shows a definite rise to a maximum immediately before convulsions. During convulsions there is a fall in the respiratory quotient. The severity of symptoms is associated with the magnitude in the changes in the respiratory quotient.

In decapitate cats injected with curare and insulin the changes in O_2 consumption show variations which do not follow any definite rule. The most striking change is the rise in respiratory quotient nearly to unity.

When the caloric output is calculated it is found that the total metabolism does not change in any marked degree except when convulsions occur. There is usually a fall in the caloric output just before the convulsions. The proportion of the caloric output due to combustion of carbohydrate, however, rises from zero or a low level, until just before time for convulsions it often accounts for the total caloric output. The same marked rise in the proportion of the caloric output due to carbohydrate combustion is seen following the injection of insulin in the curarized animals.

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THE RESPIRATORY EXCHANGE IN FROGS DURING MUSCULAR EXERCISE AND AFTER INJECTION OF INSULIN

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The normal respiratory quotient of winter frogs kept in the laboratory is about 0.70 (Shafer, 1924). If the metabolism of the frog is similar to that of the higher mammals this would mean that practically none of its caloric output is from utilization of carbohydrate, but all is from fat. Insulin raises the R.Q. of mammals to 1 (Olmsted and Taylor, 1926) and the experimental evidence indicates that the rate of metabolism is practically unchanged or may even be lowered by insulin, but the type of metabolism is changed in that the proportion of carbohydrate burned is increased. The following experiments were undertaken to discover if the frog's metabolism would change in a similar manner to the mammal's after injection with insulin.

In the first series of experiments 3 medium sized frogs were placed in a dry 500 cc. jar. The jar was closed by a rubber stopper having two holes, one communicating with an outlet for taking a sample of air, the other with a mercury reservoir. The frogs were left in the closed jar for 15 minutes. Then slightly more than 10 cc. of mercury was run in, and an air sample taken under positive pressure. This allows the syringe to withdraw the full 10 cc. and obviates the possibility of contamination through leakage. The frogs were immediately removed from the jar, washed in tap water at room temperature (18° to 21°C.) and returned to the battery jar in which they were kept. This slight contact with mercury did not appear to affect the frogs, nor did the concentration of CO_2 during the 15 minutes' enclosure appear to affect them, for there were no evidences of increased respiration or even restlessness. The CO_2 never reached a concentration of 3 per cent, which is the threshold at which man begins to be disturbed, in fact it seldom reached 2 per cent.

The surprisingly low R.Q.s after convulsions, and the rise within three hours to unity, made it evident that it would be far better to study individual frogs and follow their respiratory exchange at close intervals of time. Accordingly a single frog was placed in a 125 cc. jar and air samples were taken in the manner described above. In a lot of 4 insulin frogs the

R. Q. rose in one case within 3 hours to a maximum of 0.87, and in 2 others to 0.91 and 1.03 in 6 hours. The R. Q.s after 11 hours were found to be 0.82, 0.80, 1.03 and 0.98. This was a more rapid rise than was expected since as a rule convulsions do not usually appear until after 20 hours. Another lot of 5 frogs injected with a different make of insulin, however, gave similar results, i.e., 10 hours after insulin the R.Q.s were 0.94, 0.99, 0.102, 1.07, 1.01. Examples of a more delayed effect are seen in figure 1.

The following protocol shows a typical record:

	PER CENT CO_2	PER CENT O_2	R.Q.
October 30. Lot 1—3 frogs....	1.40	1.86	0.75*
October 31. Lot 1	1.34	1.88	0.74
Lot 2	1.44	1.90	0.76
Lot 3	1.18	1.68	0.70
Lot 4	1.57	2.28	0.76
November 2. Lot 1	1.25	1.71	0.73
Lot 2	1.46	1.96	0.74
12 m. each frog of lots 1, 2, 3 given 1.5 units insulin			
2:30 p.m. Lot 1	2.00	2.56	0.78
3:15 p.m. Lot 2	1.20	1.64	0.73
3:45 p.m. Lot 3	1.50	1.96	0.77
November 3. 9 a.m. Convulsions going on in all 3 insulin jars.			
11 a.m. Lot 2 quiet	0.95	1.61	0.59
11:30 a.m. Lot 3 quiet	0.45	0.67	0.67
11:45 a.m. Lot 4 (control)	1.16	1.62	0.72
12 n. Lot 3 quiet	1.38	2.57	0.50
2 p.m. insulin frogs limp, practically no movements, sense of equilibrium gone, in majority			
2:45 p.m. Lot 1	2.02	1.96	1.03
3 p.m. Lot 3	1.07	1.08	1.00
8 p.m. Lot 1	1.60	2.06	0.78
8:30 p.m. Lot 3	0.93	1.04	0.84
8:45 p.m. Lot 4 (control)	1.00	1.41	0.71

* Temperature 19.50°C.

In the experiments quoted above the respiratory exchange was never taken while the frogs were actually in convulsions, and the highest R.Q. reached under these conditions was 1.07. To follow the changes just preceding and during convulsions without having to handle the frogs, which usually brings on convulsions prematurely, the frog to be examined was placed in a 125 cc. chamber through which passed a gentle stream of air saturated with water vapor. When a gas sample was to be taken the air

supply was shut off and the frog breathed in the closed chamber for a definite time, usually 10 minutes. A removal of sample, the mercury was drawn off at once and the air was turned on so as to pass vigorously through the chamber for a few moments, then regulated to the usual gentle stream. The whole procedure caused little disturbance to the frog. Normal frogs left in the chamber for 24 hours appeared to be none the worse for the treatment. The R.Q.'s of normal frogs under these conditions were 7.0, 6.8, 6.8, 7.2, etc.

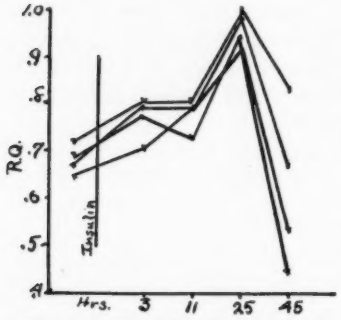

Fig. 1. R.Q. of insulin frogs kept at 19°C.

The following is the record of an insulin frog which did not have sufficient insulin to produce convulsions.

Before insulin, per cent CO_2 = 1.69, per cent O_2 = 2.33, R.Q. = 0.72, Tot. cal. hr. 0.0536, Cals. due to carbohydrate 0.0025. 2½ units insulin under tongue.

	PER CENT CO_2	PER CENT O_2	R.Q.	TOTAL CALS./HR.	CALORIES DUE TO CARBOHYDRATE
13 hours after insulin	1.22	2.06	0.60		
15½ hours after insulin	0.75	0.91	0.83	0.022	0.0093
18 hours after insulin	0.81	0.92	0.88	0.0225	0.0110
18½ hours after insulin	0.50	0.53	0.95	0.0138	0.0114
20 hours after insulin	0.64	0.61	1.05	—	—
22 hours after insulin	0.50	0.50	1.00	0.0136	0.0136
4 days after insulin	1.50	2.01	0.75	0.0473	0.0071
5 days after insulin	1.60	2.14	0.75	0.0478	0.0071

If we may assume that the frog's metabolism is similar to that of the mammal, then using Lusk's tables we can calculate the caloric output per hour. The volume of air in the jar with the frog inside and the rubber stoppers in place was approximately 85 cc. The animal was allowed to breathe for 10 minutes in this closed space. Therefore, the per cent of O_2 used $\times$ 85 $\times$ 6 = the volume of O_2 used per hour. In this way the figures in the last protocol were obtained. It will be seen that while insulin is having its effect the actual metabolic rate expressed in calories per hour is lowered to one-fourth the normal value, and after the effect has worn off there is a return toward the normal again.

If, however, the frog has vigorous convulsions, and an air sample is taken at this time, the R.Q. proves to be very high.

February 15. 3:30 p.m. 2 units insulin under tongue

February 16. 10:30 a.m. Frog O.K. Not hypersensitive. Placed in respiratory chamber.

11:30 Restless, wipes face frequently. 104 breaths per minute. Per cent $CO_2 = 1.52$; per cent $O_2 = 1.58$; R.Q. = 0.96.

1 p.m. Quiet, breathing rather shallow, 60 breaths per minute.

2 p.m. per cent $CO_2 = 0.79$; per cent $O_2 = 0.79$; R.Q. = 1.00.

4 p.m. Sense of equilibrium going. Opens mouth and protrudes tongue. Skin paler. Breathing irregular, not more than 12 per minute.

4:30 p.m. Violent convulsions. Air sample taken during convulsions. Per cent $CO_2 = 1.60$; per cent $O_2 = 0.96$; R.Q. = 1.67.

5 p.m. Sitting up. Breathing 102 per minute.

February 17. 10 a.m. Breathing 120 per minute. per cent $CO_2 = 1.20$; per cent $O_2 = 1.40$; R.Q. = 0.86.

3:30 p.m. Breathing 50 per minute. Per cent $CO_2 = 0.35$; per cent $O_2 = 0.47$; R.Q. = 0.74.

The high R.Q.s of 1.67, 1.35, etc., during convulsions were followed by very low ones, 0.47, 0.50 (see also fig. 1). These changes and the changes in breathing indicate that the muscular effort during convulsions must be taken into account when considering the effects of insulin on gaseous exchange. To determine the part played by muscular contraction we arranged to stimulate a frog in the respiratory chamber with maximal induction shocks sent in at the rate of 2 per second. Ten experiments all gave results similar to those seen in figure 2. During stimulation the R.Q.s rose to such high values as 1.62, 1.56, 1.50, 1.45, etc. The rate of respiration was always lowered, but much deeper breaths were taken, sometimes a frog even opened its mouth when emptying the lungs. The volume of CO_2 given out during the period of stimulation was several times the normal, in one case $7\frac{1}{2}$ times the normal and in this same case the O_2 consumption was $3\frac{1}{2}$ times the normal.

After exercise the CO_2 output and O_2 used both decrease, but the CO_2 falls much more rapidly, hence the R.Q. sinks to such low values as 0.42 and 0.45. The fall in the R.Q. and its subsequent rise to normal took just over four hours in two fairly small frogs weighing 25 gm. each, which had been stimulated for exactly $7\frac{1}{2}$ minutes (the record of one of them is shown in fig. 2). The CO_2 and O_2 both fell to normal in about an hour. Instead of the O_2 consumption remaining above normal as one would expect if the frog ran an oxygen debt in a manner similar to man (Hill, 1926), both the CO_2 and O_2 fell below the normal. When the R.Q. returned to normal the total gaseous exchange was reduced to nearly one-third of its original value: in man it is higher than before the exercise. Calculated as calories per hour this meant in one case a decline from 0.0274 to 0.0079 calorie per hour, although the R.Q. was the same for both.

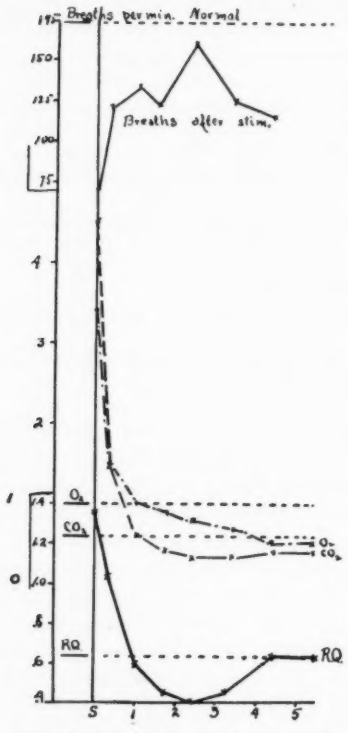

Fig. 2. Respiratory exchange of frog before, during and after muscular exercise. Normal level of breathing, per cent O_2 and CO_2 and R.Q., shown at left. *S.* = stimulation for 7½ minutes. Abscissae = hours after stimulation.

The parallel between the respiratory exchange during muscular exercise and during insulin convulsions is evident. The R.Q. rises to the same high level, but because of the depressing effect of insulin on the total metabolism of the frog causing the caloric output to be reduced from one-half to one-fourth its normal value, the actual volume of CO_2 given out during convulsions may be no more than that given out in the normal respiration of a normal frog, and yet be double that given out by an insulin frog in the period just preceding convulsions. The O_2 consumption during convulsions is always very low, less than in normal respiration.

The total gaseous exchange after insulin convulsions is very low indeed. Often the frog becomes perfectly limp and no respiratory movements are to be seen for half an hour at a time. Under these conditions it is necessary to leave the frog in the respiratory chamber 20 minutes to half an hour to obtain enough CO_2 to make the determinations. In such cases the amounts of CO_2 and O_2 reported have been reduced to the value they would have had over a 10-minute period, for purposes of comparison.

CONCLUSION

The following conclusions seem warranted from these results. In the winter frog kept in the laboratory at room temperature insulin depresses the general metabolic rate as measured by the gaseous exchange, while changing the type of metabolism from combustion of fat to the combustion of carbohydrate. If convulsions do not appear the R.Q. does not rise significantly above unity. If, however, convulsions appear the R.Q. may rise as high as that of a normal frog during severe muscular exercise,

though the total gaseous exchange is on a distinctly lower level quantitatively. Following this high R.Q., a very low R.Q. is to be observed both after insulin convulsions and muscular exercise. In a 25 gram frog exercised for 7½ minutes, the R.Q., high during the exercise, fell below the normal, then rose again to normal in just over 4 hours. But after the first hour both the CO_2 output and O_2 consumption were distinctly lower than before the exercise, and below the average normal exchange. Hence the frog does not make up its oxygen debt by using an excess of O_2 over the normal for a given period as man does, but rather his total metabolism is lowered, the CO_2 output more than the O_2 consumption.

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ABNORMALITIES OF POSTURE AND PROGRESSION IN THE PIGEON FOLLOWING EXPERIMENTAL LESIONS OF THE BRAIN

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The act of walking may be divided into two components; one postural, a contraction of the extensor muscles overcoming the force of gravity and enabling the animal to stand, the other progressive, achieved by alternate rhythmic flexion and extension of the legs. Normally these components are nicely coördinated but Sherrington (1896), (1898) has demonstrated that after transection of the brain of a mammal in the region of the mesencephalon the postural element becomes accentuated and a strong contraction of the antigravity muscles ensues. With the postural reflex dominant, progression is inhibited so that flexion of the limb occurs only after the application of a maximal stimulus. Sherrington (1910), Forbes (1912) and Graham Brown (1912), (1914) have further shown that progression is a fundamental activity of the central nervous system presided over by two balanced antagonistic centers, one initiating flexion, the other extension of the limb, now one center, now the other becomes dominant leading to coördinated alternate rhythmic movements.

The development of walking reflexes in young animals has recently been studied (Weed, 1917; Langworthy, 1924). The postural reaction is not yet demonstrable in new-born kittens and rabbits but appears at an early period in extra-uterine life. Progressive activity is accentuated in these new-born preparations after decerebration but coincident with the development of extensor rigidity in older preparations, progression is inhibited. Young guinea pigs, on the other hand, animals born when very mature, all show extensor rigidity after decerebration, with no progressive tendency.

Experiments upon pouch-young opossums demonstrate the course of maturation of the centers for both progression and posture (Weed and Langworthy, 1925a, 1925b; Langworthy, 1925). New-born opossums have well-developed fore-legs and are able to climb into the pouch by grasping the hair with the claws. The hind-legs are in an early stage of differentiation. Once inside the pouch the young become firmly attached to the nipple and activity is definitely limited. During the first fifty days

of life there is little coördination between the fore- and hind-legs. Only in somewhat older animals, in whom the eyes are open, do perfectly coördinated rhythmic movements of the extremities occur.

Opossums less than fifty days old after decerebration showed reactions similar to those of the intact animal although less active. In one specimen, fifty-six days old, the progressive activity became accentuated after the operation and walking movements continued for over an hour. Slightly older opossums were also active but walked on greatly extended legs—the postural component was apparent for the first time and the postural and progressive tendencies seemed balanced. Older preparations developed such strong extension of the legs upon any attempt at walking that they could no longer balance their weight. Later, extensor rigidity became demonstrable in the legs. The reactions of adult decerebrate opossums differed from those observed in cats and dogs in that the extensor rigidity was interrupted at intervals by flaccid flexion of the limbs or by progressive activity.

Attempts to ascertain the central nuclei and tracts involved in walking movements have made it plain that large portions of the central nervous system are directly concerned in their coördination. The accentuation of posture seen after decerebration is considered the result of release from the control of higher centers. Weed (1914) demonstrated by electrical stimulation that the inhibitory fibers descend to the mid-brain through the fronto-pontine tract. Warner and Olmsted (1923) later showed that the removal of the frontal lobe in the adult cat caused abnormal extension of the legs. Camis (1923) recently confirmed the observation of Weed (1914) that the cerebellum is an important if not essential link in the postural reflex. A cat with its spinal cord transected in the upper cervical region can no longer support its weight upon its legs, showing with certainty that essential postural centers must lie in the brain but the exact position of the centers in the mesencephalon or rhombencephalon is still disputed. Laughton (1924) found that at least two-thirds of the thalamus must be intact in the adult cat if spontaneous progressive movements were to occur. Weed (1917) had previously shown that new-born kittens after decerebration exhibited prolonged progressive movements. It appears, therefore, that in the maturing of function the control of progression is assumed by higher centers in the region of the thalamus.

Our present information concerning the physiology of the walking reflex has largely been obtained from the study of mammalian forms. It would seem, however, that in all animals which support their weight and walk upon legs, similar centers in the central nervous system must be operative. Posture and progression are now studied as evidenced in certain examples of the premammalia in the hope that the central reflex mechanism might be revealed in simpler form or that anomalies might

appear which would throw light upon the general problem. There is little previous experimental work which deals with this subject.

The author has at one time studied the central control of locomotion in fish. The dogfish (Squalas acanthus) was the object of experimentation at the Marine Biological Laboratory at Woods Hole during the summer of 1923 to test the effect of transections of the brain upon the normal swimming reaction. It was found that removal of the fore-brain and mid-brain had no effect upon swimming. Moreover, after the spinal cord was cut in the cervical region swimming reactions were perfectly normal. Locomotion in the dogfish, therefore, can be perfectly coördinated by centers in the spinal cord without the intervention of the brain.

The alligator, an example of the reptilia, walks much of the time with the belly close to the ground but often the legs, particularly the fore-legs, are much extended. Bagley and Richter (1924) showed that an excitable area exists in the cortex of the alligator, stimulation of which causes movement, not of one extremity but usually of both contralateral legs; the tail is at the same time pulled to the opposite side. Later it was shown that if the fore-brain and mid-brain were removed changes in posture and progression occurred (Bagley and Langworthy, 1926). The alligator attempted to walk but with the first few steps the legs became more and more extended until the extension of the fore-legs was maximal. The abnormal posture appeared to inhibit progression and the preparation would stand quiet in this position for a long time. Upon the application of a strong stimulus the alligator was induced to walk but with the first step it fell to the side. When quiet the limbs were normally flexed and no tendency to rigidity was apparent. After removal of the midbrain in the alligator, then, the postural reflex became accentuated upon any attempt at walking and progressive activity was at the same time inhibited.

In the present paper, the control of posture and progression in birds will be discussed: the pigeon has been utilized for these experiments. Avian forms diverge, it is true, from the direct line of phylogenetic development but the small size of the cerebral cortex and the great importance of the corpus striatum in birds offers a unique opportunity for experimental ablation of this structure with a view to studying its participation in motor activity. Birds not only show well-developed postural and progressive reflexes but the long-legged birds particularly display extremely precise postural adjustment.

Since the time of Rolando and Flourens observations have been made of the behavior of pigeons after the removal of portions of the central nervous system. The most extensive recent work is that of Rogers. He found that electrical stimulation of the cerebral cortex of the pigeon gave rise to few reactions that could not be interpreted as spread to lower centers (1922a). Rogers (1922b) removed portions of the corpus striatum and studied

changes in the normal cycle of reflex activity over long periods of time. Careful controls of the lesions both grossly and microscopically were made. In Rogers' decerebrate preparations both hemispheres were removed leaving the thalami intact. The behavior of these pigeons was characterized by equilibratory disturbances, listlessness and occasional forced flying movements. The forced flying movements he thought occurred only when the thalamus was damaged. Further experiments strongly suggested that periods of restlessness seen in the decerebrate animals might be correlated with motor activities of the digestive tract (Rogers, 1916).

Although Rogers did not report particularly concerning the central control of posture and progression, he revealed anomalies in sympathetic control after removal of the hemispheres which, in the bird, are composed largely of the corpora striata,—these experiments must be considered in comparison with the more recent work of Hunter (1924a, 1924b). Thus Rogers confirmed older observations that after removal of the hemispheres the feathers tended to be ruffled indicating a functional tone relationship between penna-motor nerves and the basal portion of the cerebral hemispheres (Rogers, 1921). Stimulation of areas in the base of the cerebral hemispheres led to the characteristic contraction of the muscles that flatten the feathers (Rogers, 1922a). Removal of the cerebral hemispheres also caused a permanent slight fall in blood pressure. The cerebral hemispheres appear to exert a continuous tonic influence upon the mechanism whereby arterial tone is maintained (Rogers, 1920).

Martin and Rich, in 1918, studied the activity of decerebrate and decerebellate chicks. An opening was made in the skull and portions of the brain removed with a pipette. These authors distinguished three types of decerebration—shallow when only the pallium was removed, standard when both hemispheres were completely extirpated and deep when the thalamus was also injured. After shallow decerebration, the chicks seemed to have less initiative and to be less wild. In the standard group, locomotion was normal but the birds were drowsy and the scratching reflex was poorly executed. After injury to a portion of the thalamus, a tendency to extensor rigidity was noted in the legs of two, walking was unsteady and muscular weakness apparent.

After the present work was already well begun Hunter (1924a, 1924b) published the results of experimental removal of the corpus striatum upon the normal posture of birds, the sea gull and domestic fowl. He believed that the anomalies described were due to the release of sympathetic plastic tone from the normal control of centers in the corpora striata. Hunter postulated two distinct types of tone in muscle as follows:

> Contractile tone, subserved by the somatic arc, acts on certain muscles only, while their antagonists are reflexly inhibited. This element of tone, therefore, imposes a posture on the part. Plastic tone, a function of the sympathetic arc, maintains

this posture once assumed by maintaining the length of the fibers of the muscles which exhibit contractile tone.

Hunter removed the corpus striatum on one side after the cerebral cortex had been turned toward the midline leaving it in a theoretically functional condition. The operation produced rigidity of all the limbs and standing could no longer be evenly maintained. In the seagull there was a tendency to rise on the toes constantly and no single position was maintained for long. The wings were also rigid and were sometimes maintained in a position higher than usual so that the adjacent borders were almost in contact. The rigidity maintained its intensity during passive movement and the wings remained fixed in any new position assumed even when this position was so abnormal that the fibrous structures were put upon the stretch. Both wings exhibited this phenomenon after unilateral removal of the corpus striatum but the rigidity was greater on the ipsilateral side brought out clearly upon attempted flight when the bird was held captive in the hand. A greater effort was required to carry the wing through a smaller range of upward movement than on the less affected side. An increased effort marked the downward movement also and the wing seemed to overshoot the position at which it normally ended. The flight of the birds was in a circle toward the injured side.

Hunter described a like condition after cutting the somatic fibers to the wing, removing contractile tone and leaving sympathetic plastic tone accessible for independent study. He believed that increased plastic tone in both the flexor and extensor muscles of the wing was responsible for the diffuse muscular rigidity which followed removal of the corpus striatum in birds and that this was remarkably similar to the Parkinsonian rigidity in man. Conversely, Hunter found that when the sympathetic fibers innervating the fowl's wing were cut the wing hung lower on the operated side but after a few days this drooping and abduction were less marked. The abnormality was more clearly demonstrable after fatigue of the wing musculature.

Kappers' (1922) morphological studies are quoted by Hunter in support of the sympathetic function of the corpus striatum. Kappers has shown that the corpus striatum develops near the frontal end of the sulcus limitans which, according to Herrick, ends in the pre-optic recess. He suggested that the corpus striatum might be of sympathetic origin.

Hunter's experiments upon birds were aided by a study of the classical work of Langley (1904) on the sympathetic system of birds and the muscles that move the feathers. A review of Langley's observations will also aid in the interpretation of the present work. Langley showed that movement of the feathers in birds was an easily observable effect of stimulation of sympathetic nerves; these movements also occurred after curare had been injected, showing that the effect was not due to stimulation of somatic

nerves. Stimulation of the spinal cord also caused movements of the feathers, usually a depression but sometimes an erection similar to that seen when a bird ruffles his feathers. This effect was also obtained by stimulation of the brain from the thalamic region downward. Langley did not observe it after stimulation of the cerebrum or cerebellum. Moreover, strychnine or asphyxia did not cause movements of the feathers after the preganglionic fibers were cut.

After section of the preganglionic fibers to a given spinal nerve the feathers innervated by this nerve were less depressed than normal. This condition increased for a day or two, then slowly subsided and disappeared. It would appear that normally the central nervous system exerts an effect upon the muscles of the feathers. The degree of erection after cutting the autonomic fibers varied in different parts of the body, being well marked over a small area upon the head just above the beak and distinct in the back of the neck. On smoothing down the feathers they slowly rose again over the injured area.

Section of the spinal cord in the pigeon caused rhythmical erection and depression of the feathers over areas innervated below the lesion. This erection of feathers when marked was too strong to be due to inhibition of muscular tone. Langley found that some muscles attached to the feathers were definitely erectors, others depressors and that the depressors were nearly always stronger. These findings probably explained the predominance of depression on nerve stimulation. Both erector and depressor muscles are innervated by sympathetic nerve fibers and are stimulated to contract separately.

Technique of experiments. Twenty-one adult pigeons were utilized in this series of experiments. Most of the observations were continued only for a period of a day during which time a number of different procedures were carried out upon the same preparation. A few of the operations were done under sterile precautions and the birds kept alive for weeks. It is needless to say that ether was administered during each operation. Intra-tracheal anesthesia was employed since it was found more satisfactory than administration of ether by cone. In the bird a small rubber catheter may easily be inserted in the trachea.

After cutting the feathers over the top of the head an incision was made in the midline and the skin flaps turned to the sides. The bone was carefully removed over one hemisphere with care to keep the dura intact; the dura was then carefully opened by means of a hook and sharp scissors exposing the surface of the hemisphere.

The exposed cortex in a number of animals was explored for electrically excitable areas to confirm the results of Rogers and workers who preceded him. Both stigmatic and bipolar methods of stimulation were employed. The stigmatic electrode has obvious advantages, minimizing as it does the

possibility of spread of current and trauma to the cortex. But bipolar stimulation has been largely employed by previous observers. The method of stimulation and depth of anesthesia play an important rôle in determining the extent of excitable areas. It is imperative, therefore, in comparative studies that the same methods be used throughout.

One or both hemispheres were then removed with a blunt spatula. The bird was allowed to recover from the results of the ether and its reactions studied. Later in the day the thalami and finally the midbrain were removed in a similar manner. Bleeding was controlled by the application of cotton pledgets.

EXPERIMENTAL OBSERVATIONS. *Electrical stimulation of the cortex.* The results of stimulation of the cortex confirm, in general, the findings of Rogers (1922a) and other earlier investigators and will, for this reason, be considered only briefly. When the pigeons were deeply anesthetized no response could be obtained. Rogers had the same experience and Bagley and Richter (1924) working with the alligator obtained similar results. In the case of mammals, it is generally customary to record only those responses obtained when the animal is well anesthetized, avoiding, as this does, the possibility of confusion with voluntary movements. The technique of workers differs greatly however, and complicates any comparison.

Even with the anesthesia light the majority of the cortical areas were silent and no movement of the extremities was obtained. Stimulation of the area designated by Rogers in the medial portion of the occipital lobe caused a wavering and sometimes a quick constriction of the contralateral pupil. Stimulation in the region of the frontal lobe caused a depression of the feathers upon the throat. Rogers felt that this was a true cortical response not due to spread of current to underlying structures. The cerebral cortex of the pigeon is a thin layer of nervous tissue showing no lamination similar to that characteristic of the neopallium of mammals. The responses to electrical stimulation are also few and it is possible by this method to determine little concerning the function of this area. Previous workers have shown that removal of the cortex without injury to the corpus striatum has little apparent effect upon the subsequent behavior of birds.

Unilateral ablation of corpus striatum or the entire hemisphere. Hunter (1924a, 1924b) in removing the corpus striatum cut the cortical layer at its lateral edge and turned the flap toward the midline thus leaving the important association pathways intact. The corpus striatum, thus exposed to view, could be removed with a blunt curette. This operation for the present research seemed to have little advantage over removal of the entire cerebral hemisphere; both types of preparations were made and the results in so far as they concerned posture and progression seemed identical.

Both operations had a marked effect upon the subsequent posture of the birds. The pigeons, when lying upon their side, before complete recovery

from ether held both legs flexed but the flexion was greater on the side opposite the lesion, this leg offering also more resistance to passive extension. When placed upon their feet the birds stood high upon their legs and leaned backward; due to difficulties in postural control they swayed in the plane of the long axis of the body. By causing the pigeons to stand first on one leg and then on the other it was easy to demonstrate that the extension was more pronounced on the same side as the lesion for upon attempts to stand on this leg alone the extension became so great that balance was impossible. This inequality was further evidenced by the fact that three of the pigeons stood continually on the leg opposite the lesion holding the other flexed close to the body. One stood upon the contralateral leg for two hours and a half nor could it be persuaded to stand on both; such an attempt caused at once loss of balance and forced flying movements. These three birds leaned toward the unoperated side and turned the head to that side in the attempt to maintain equilibrium.

Several of the pigeons learned backward far enough to support their weight upon their tail feathers. When these were cut close to the body one leaned still further till it balanced on the stump. The neck was extended so that the birds gave the appearance of looking at some high point. In this position the ruffling of the feathers of the throat, later to be described, made the preparations simulate the appearance of pouter pigeons. When the body was tipped to a normal angle the hyperextension of the legs became more marked; the weight then was supported on the tips of the claws. On a perch the birds fell backward and had to be continually righting themselves.

The preparations differed from those in which both hemispheres had been removed in that the latter were alert and active, keenly responsive to every movement and noise. They appeared to walk as well as ever, the postural abnormalities not interfering when locomotion was attempted. The pigeons when held in the hand kept the legs flexed, the toes were often tightly flexed. There was, therefore, no extensor rigidity in the legs: indeed it seemed at times that some increased flexor tone was present.

Little change was ever noted in the position of the wings which were possibly held somewhat higher than normal. There was no constant difference in the height of the two wings. Moreover, in contradistinction to the findings of Hunter we would lay particular emphasis upon the fact that there was no tendency for the wings to remain fixed in any position in which they were placed passively. The birds flew apparently normally, not deviating to one side or the other.

The feathers tended to be ruffled after the operation; this erection was bilateral but often more marked on the same side as the lesion. All portions of the body did not show this equally, the effect being most marked on the throat and neck and the caudal portion of the back. It was most

evident when the pigeons were quiet and undisturbed; when the birds walked the feathers became sleek. Indeed, this difference between the condition of the feathers in the alert and quiet preparation was very striking.

Ablation of both cerebral hemispheres. The reactions of birds with both hemispheres removed differed markedly from those already described for instead of preparations as responsive as normal pigeons, the animals were now listless and lethargic. If undisturbed, they would stand for a long time in one position not responding to loud noises or resisting handling. When picked up these birds with hemispheres removed became temporarily excited but almost immediately became quiet again. If placed upon the side they would remain still and make no attempt to walk. Periods of restless activity at times occurred; Rogers believed that they were due to the motor activity of the digestive tract, the result of hunger.

Definite difficulties in posture were very evident. Balance was not well maintained due to increased extension of the legs so that the animals tended to fall backward and to overcome this unsteadiness swayed backward and forward, teetering upon their legs. When in real danger of falling they became aroused for a moment from their listless condition and adjustment became temporarily more perfect. Some of the birds overcame this postural difficulty by crouching upon semi-flexed legs. No resistance to passive flexion of the extremities was ever noted. Indeed when lying on the side the legs were always held flexed. Aroused from their lethargy by strong stimulation the pigeons walked quite normally but they only took a few steps and then became quiet and unresponsive again.

The feathers were extremely ruffled when the birds were undisturbed—a phenomenon noticeable particularly over the neck and throat but marked over the body and extremities as well. At any attempt at spontaneous movement the depressor muscles contracted and the feathers lay smooth and sleek. When cold the birds shivered.

Cerebral transection below the thalamus. Birds with the thalamus removed no longer attempted to stand but lay on the side with the legs flexed close to the body. No postural reflex seemed active and if placed upon their feet their legs immediately gave way. Increased flexor tone was present for considerable force was required to extend the legs. The pigeons, although quiet much of the time often struggled, making flying movements which were incoördinated but were so violent that if unrestrained the birds beat themselves against obstructions. As Rogers (1919) has already shown, removal of the thalamus destroys in the bird as in mammals the mechanism for heat regulation. The pigeons no longer shivered when cold as did the birds with both hemispheres removed. They were, therefore, kept in a warm box at a constant temperature.

Transections below the mid-brain. The cut in this case was made just

cephalic to the anterior surface of the cerebellum. There was always considerable bleeding and several of the preparations developed irritative symptoms, jerking and twisting the neck and ventral bowing of the back; these reactions could be easily recognized.

After the operation, the birds could no longer stand but lay on their side with legs always flexed. Resistance to passive extension of the legs was not as great as in pigeons with the midbrain intact. They remained quiet and no forced flying movements occurred.

From this group a very definite postural reflex could be elicited. If the claws were held firmly upon the table and an effort made to balance the weight upon the legs they assumed by a series of jerks a position of extreme and maximal extension. Indeed, so great was this extension that the body weight was no longer balanced and the pigeon immediately fell backward. If the weight was not supported upon the legs the extension was instantly lost and the legs assumed a position of passive flexion. By grasping the beak and gently pulling the neck forward a few progressive beats of the legs were obtained. No extensor rigidity of the legs was ever noted, indeed at rest the legs always assumed a flexed position. The extension noted after removal of the midbrain was greater than ever observed when the hemispheres alone were removed. The latter preparations were able to stand voluntarily and the hyperextension was, therefore, more noticeable; in the former group, the extension could be elicited only by passive balancing of the body weight. The feathers tended to stand erect upon the body after the manner already noted in the previous preparations.

Transection of the spinal cord in the thoracic region. The spinal cord in two pigeons was exposed under sterile conditions and severed in the thoracic region. One of the birds was killed at the end of five, the other after fifteen days. Little shock was produced by the operation and as soon as the birds recovered from ether, pinching of the claws caused flexion of the ipsilateral leg and extension of the contralateral. If the feet were placed in contact with a solid surface rhythmic progressive movements of the legs occurred but all control of posture was lost, and the pigeon could no longer stand. Nor did any postural reflex return during the period of observation.

The feathers innervated by the spinal cord below the lesion were ruffled, particularly those upon the back. Pinching the claws made this erection more noticeable and caused the feathers to stand almost straight out from the body. When quiet the ruffling was evidenced to a less degree. This phenomenon was most marked for the first two or three days after the operation.

Discussion. These neurological findings in the pigeon are indeed difficult of correlation and interpretation for we have not here the background of physiological research that aids us to understand the reactions

of mammalian forms; and in addition the morphological studies of the bird's brain are likewise incomplete. Nevertheless, we feel that it is possible to lay emphasis upon a few general conclusions.

Progression in the pigeon as in the mammal appears as a fundamental activity of the spinal cord, for after transection of the thoracic portion of the spinal cord alternate, rhythmic, progressive movements of the legs occurred when the claws were placed in contact with a solid surface. But the pigeon could never support its weight upon its legs:—posture, as in the mammals is controlled by the brain.

The wide participation of the brain in the control of walking movements is striking. After removal of one or both hemispheres and again after transection below the mesencephalon, accentuation of the postural reflex has been observed in pigeons. When a cut was made below the level of the thalamus the bird no longer was able to stand upon its legs. The complicated morphological pathway for the control of walking movements in mammals has been mentioned in the introduction; here we see that it holds equally true for avian forms.

In mammals the extensor rigidity appearing after transection of the mesencephalon is considered as evidence of a release from the control of higher centers. Warner and Olmsted (1923) have shown that after removal of the frontal lobes in the cat, the limbs become abnormally extended although if the motor areas are left intact, well executed walking movements occur. Indeed, as Laughton (1924) has shown, in the cat with the lower two-thirds of the thalamus uninjured spontaneous walking is possible. It is only when nuclei just above the midbrain are rendered functionless that the remarkable phenomenon of decerebrate rigidity appears in mammals.

In an analogous way accentuation of posture is seen in the pigeon after the removal of one or both cerebral hemispheres. If this is a phenomenon due to release from inhibition we must postulate the presence cephalad to the lesion of a strong controlling postural center exerting its effect bilaterally but preponderantly on the same side. This motor center evidently does not lie in the cortex which in the bird is only slightly developed, does not show the lamination characteristic of mammals nor upon stimulation yields well-defined motor reactions. Indeed, the same physiological injury is seen when the cortex is turned medially in a theoretically undamaged condition and the corpus striatum alone removed. The corpora striata in the pigeon, therefore, seem in a measure to control the postural reflex.

Even greater extension of the legs is observed after removal of the mesencephalon. It will be remembered that abnormalities in posture in the alligator were not observed until after removal of the midbrain. In both reptiles and birds we find in the superior colliculi an important reflex center

influenced strongly by the entering optic fibers. Large cells of a morphological motor type lying in this area send axones to end in the medulla and spinal cord. It may be that these cells exert an important control over posture which is released after removal of the midbrain. Extension in the alligator was accentuated upon any attempt at walking; the bird after the thalamus is removed no longer stands voluntarily and extension is only made observable by balancing the body weight with the claws held upon a solid surface.

In the pigeon as in the alligator, in contradistinction to all adult mammals previously studied, no continuous extensor hypertonus was ever noted and hyperextension was only demonstrated upon attempts at standing or walking. When quiet the legs were held in passive flexion. Young mammals, opossums, after decerebration, behave in a similar manner at a certain period in their development. They walk upon maximally extended legs but when quiet the legs are flexed and flaccid. Somewhat older animals manifest extensor rigidity and progressive activity is inhibited.

The operated pigeons not only held the legs flexed when lying on their side, but in many of the preparations, particularly those with the brain transected below the thalamus, flexor tone seemed increased. Birds during flight hold the legs flexed close to the body. As we find centers in the brain controlling the antigravity muscles so we would expect, particularly in animals with special need for such adaptation, to find encephalic centers controlling flexor tonus.

Many of Hunter's (1924a, 1924b) observations upon the bird have not been confirmed in this work. No clear-cut abnormalities in the position of the wings after removal of one or both cerebral hemispheres were seen, nor gross incoördination of flying movements. The wings would not remain fixed in position when passively placed in abnormal attitudes. In the experiments described we find no thesis for the theory of plastic tone; the postural reactions described seem quite analogous to those already described in mammals. That decerebrate rigidity is subserved by the somatic rather than by the sympathetic reflex arcs is the opinion of the majority of investigators today. Coman (1926) has recently published a critical review of previous work upon this subject. He has, moreover, shown that no extensor rigidity will develop in the fore-limbs of a cat or dog after severing the somatic arc even though the sympathetic reflex arc is still intact.

The ruffling of the feathers after removal of the hemispheres was a striking result of these experiments. It was also remarkable that they became flattened and sleek when the animal was alert and were ruffled again when the bird was quiet. After section of the spinal cord in the thoracic region the feathers stood erect behind the lesions. Sensory stimulation in this case seemed to make them stand even more noticeably.

SUMMARY

Alternate progressive movements of the legs occur in the pigeon after transection of the spinal cord in the thoracic region; posture as in mammals is controlled by centers in the brain. After removal of one or both cerebral hemispheres, abnormal extension of the legs was observed. This phenomenon was even more evident after removal of the mesencephalon. This extension appears analogous to the accentuation of the postural reflex seen in mammals after decerebration.

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THE INFLUENCE OF BURNS ON EPINEPHRIN SECRETION[1]

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In delayed death from burns, the adrenals show characteristic changes such as focal exhaustion of the lipoids and necrosis in both cortex and medulla (1). When animals were killed within twenty-four hours after burning, Lattes (2) found no changes in the cortex except hyperemia and occasional hemorrhages. Likewise there were no marked changes ten to fifteen days after burning. Weiskotten (3) also found in man that the changes in the adrenal were not marked if death occurred a few hours after the burns.

We have been able by means of the completely denervated iris in cats, to demonstrate an increase in the epinephrin output within two or three minutes after burning (animal anesthetized by ether). This increase persisted for several hours in some instances.

The superior cervical ganglion was removed at the first operation and several days later the ciliary ganglion was removed. The experimental observations were made preferably within a day or two following the second operation because the denervated iris gradually relaxes as time goes on, so that it becomes of little use as a test object.

Five cats were used in this experiment. All were under the influence of an anesthetic. Ether was usually employed. Urethane was used in one experiment.

A difficulty which could not be avoided was the dilatation of the pupil due to ether. This make the iris less sensitive, i.e., the greater the dilatation from whatever cause, the larger is the amount of epinephrin required to produce the same increase in diameter. In some instances ether was discontinued after the completion of the experiment, in order to determine how long the increased epinephrin output continued. Adrenals were fixed in formaldehyde and $K_2Cr_2O_7$, then sectioned with a freezing microtome. The lipoids were stained with Sudan III.

In the first experiment, the size of the pupil with the animal under ether and urethane was 3.55 mm.

Burning the skin (2.5 sq. cm.) superficially with a hot rod produced no effect.

[1] Aided by a grant from the Elizabeth Thompson Science Fund.

Burning the skin more deeply and over a larger area (12 sq. cm.) caused dilatation of the pupil to 5.83 mm. in 70 seconds. At the end of 7 minutes the pupil was still dilated (4.32 mm.).

Later (cat under ether anesthesia throughout the experiment) dipping the left leg as far as 5 cm. above the heel into water at 100°C. for about 3 seconds caused the pupil to dilate to 12.43 mm. in less than 1 minute. The dilatation subsided gradually so that at the end of 6 minutes it was 5.08 mm. A slight handling of the dipped leg increased the pupil again to 6.09 mm. (anesthesia complete throughout experiment). Fifteen minutes later both hind legs and the hind quarters were dipped in water at 100°C. for 3 seconds. The pupil again became greatly dilated (12.43 mm.). The adrenals were removed and fixed within 35 minutes. The whole experiment had lasted about one hour.

Judging from the staining with bichromate, epinephrin was present in patches in the medulla and was absent or very scant elsewhere. Lipoids were absent in the zona reticularis, scant in the zona glomerulosa and moderately plentiful in the zona fasciculata.

In the second experiment (complete ether anesthesia), very small burns with an electric cautery caused dilatation of the pupil from 7.62 to 11.66 mm. Dipping the toes of one hind leg into water at 100°C. for 1 second caused dilatation of the pupil to 12.43 mm. Later, dipping 210 sq. cm. of the posterior part of the animal into water at 100°C. for 1 second caused dilatation to 12.68 mm. which was maximal. The pupil was dilated for three and one-half hours. Soon after this it returned to normal and finally became abnormally constricted so that at the end of five hours it was reduced to 2.03 mm. (6.60 mm. was normal).

The adrenals were fixed three days after the experiment, i.e., at the death of the animal. Macroscopically, there was no congestion in the adrenals but they appeared soft and in poor condition. Microscopical examination showed the entire absence of epinephrin from the medulla. Lipoids were found throughout the cortex although they were scant in the zona glomerulosa and most plentiful in the zona fasciculata.

In a third animal (ether anesthesia) the hind quarters were dipped in water at 100°C. for one second. This caused a dilatation of the pupil which did not disappear until twenty-seven hours had elapsed. The animal was killed two days after the experiment.

Epinephrin was absent from the medulla. Lipoid was not limited to any one zone but the quantity was smaller than normal everywhere.

In the two remaining animals, dilatation of the denervated iris occurred after hot water burns (complete ether anesthesia) as described in the other experiments. The dilatation persisted for two hours in one animal and for four hours in the other animal.

Both adrenals were removed from these animals twenty and twenty-

one hours respectively after the hot water application. While under the influence of ether, burns similar to those in the first experiment produced no change in the pupil.

The adrenals from the first animal showed only a few patches of chromate stain in the medulla and these were light. Lipoids were very plentiful in the *zona fasciculata*, absent in the *zona glomerulosa* and scant in the *zona reticularis*.

Epinephrin appeared to be absent in the medulla of the second animal. Lipoids were present in patches in the outer zone of the cortex.

These experiments indicate that burns produce an increase in the output of epinephrin which may persist for hours. This is associated with a disappearance of chromate staining in the medulla and a depletion of the lipoids in the cortex.

SUMMARY

Experiments on anesthetized cats show that burns cause an increase in the epinephrin output (dilatation of completely denervated iris). The increased output sometimes persists for a few hours.

There was depletion of epinephrin and of lipoids in the adrenals of these animals.

Therefore, burns cause an excessive activity of the adrenals.

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THE RÔLE OF THE SUPRARENAL GLAND IN THE NATURAL RESISTANCE OF THE RAT TO DIPHTHERIA TOXIN

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The fact that suprarenalectomized animals succumb more readily than normal animals to the effects of various toxic agents suggests that the suprarenal glands play an important rôle in natural immunity. At the suggestion of Dr. David Marine of the Montefiore Hospital, New York City, we have commenced an investigation of certain phases of this problem. This paper presents the results of an attempt to determine the influence of suprarenalectomy upon the mechanism of resistance to diphtheria toxin. Rats were selected for this work because of their great natural resistance to diphtheria toxin and their ability to survive double suprarenalectomy. Our special problem is concerned with the difference between suprarenalectomized and normal rats in respect to the lethal dose of diphtheria toxin and the disappearance of toxin from the blood.

Lewis (1923) showed that double suprarenalectomy increased the sensitiveness of rats to a variety of poisonous drugs, cobra venom, adrenalin and diphtheria toxin. Camus and Porak (1913) found a greater sensitiveness to strychnine and curare in rabbits immediately after suprarenalectomy. Marie and Morax (1914) induced tetany in guinea pigs shortly after double suprarenalectomy with a dose of tetanus toxin harmless for controls. Scott (1923) confirmed but Stewart and Rogoff (1922) were unable to substantiate the results of Lewis as regards morphine.

The resistance of the white rat to diphtheria toxin has been a matter of common knowledge. Lewis found that a dose of 200 M.L.D. for a guinea pig, while lethal for suprarenalectomized rats, had no effect upon the normal rat. Coca, Russell and Baughman (1921) reported that although rats usually survived the injection of 1000 M.L.D. they were not absolutely immune to diphtheria toxin since they regularly succumbed to 4000 M.L.D. Furthermore, they found that the resistance was not due to the presence of normal antitoxin and concluded that it was due to the property of the cells of preventing the toxin from entering them or of attaching itself to them.

Methods. The rats were obtained chiefly from the Bussey Institution of Harvard University and were a mixed stock of colored varieties of the

albino rat. A few were obtained at the Montefiore Hospital Laboratories where some of our early experiments were made. The latter were derived originally from the King strain of the Wistar Institute. The rats received a varied diet, consisting of bread and milk twice a week; a mixture of rolled oats, hominy, meatscrap, milk powder, and salt twice a week; a mixture of sunflower seed, hemp seed, and oats twice a week; and molasses horse feed once a week.

Both suprarenal glands were removed in one operation by the posterior route. The control rats received the same operative procedure except the removal of the glands. Special care was taken to produce the same amount of trauma as in the suprarenalectomized rats and sometimes a piece of fat was removed from the region of the suprarenal glands.

The post-operative care consisted of isolating the rats in individual cages protected from drafts in a warm room for several days until they had recovered their customary activity. No food was given the first day after the operation. The wounds healed rapidly, not over one per cent showing operative sepsis. Our observations agree with those of Scott that the post-operative mortality in the suprarenalectomized rats depends largely upon the post-operative care. The losses among the control animals were slight, but the mortality in the suprarenalectomized animals fluctuated markedly with the after-care, depending upon isolation, cleanliness, food and warm dry quarters protected from drafts.

Temperature regulation. The greatest mortality among the suprarenalectomized animals occurred during periods when there was an unavoidable fall in temperature in the animal house. Control rats under the same condition were unaffected. Cannon and Querido (1924) have pointed out the intimate relation of the suprarenal glands to heat regulation and it occurred to us that the susceptibility of the suprarenalectomized rats was in part due to their inability to maintain a sufficiently high body temperature when exposed to cold. This view was confirmed roughly by subjecting a limited number of suprarenalectomized and control rats to low temperatures and measuring the resulting changes in body temperature. Rectal temperatures were taken with a certified clinical thermometer before, during, and immediately after an exposure of two hours in a cold room at 3°C., and a subsequent two hours in the incubator room at 37°C. Care was taken to eliminate as far as possible errors in reading due to variations in the depth of insertion of the thermometer, the presence of fecal masses, and the struggles of the animals.

In one series three control rats had an average temperature of 99.9°F. and three suprarenalectomized rats, twenty days after operation, 99.3°F. After two hours in the cold room there was an average reduction of 0.7 degrees in the controls and 5.9 degrees in the suprarenalectomized rats. After two hours in the incubator room both groups had recovered their

initial temperature. Considerable individual variation was noted, and one rat not in this series showed marked loss of heat regulation, its body temperature falling below 85°F. in the cold room. Evidently the suprarenalectomized rats were unable to maintain their body temperatures as efficiently as the controls.

Resistance to diphtheria toxin. An attempt was made to determine the approximate lethal dose of diphtheria toxin, administered intraperitoneally, for suprarenalectomized and control rats. Two lots of toxin were used. Toxin 614 (M.L.D., 0.0025 cc.) was obtained from the New York City Board of Health and toxin 38 B (M.L.D., 0.003 cc.) from the Massachusetts Department of Public Health. Intraperitoneal injections of various amounts were given to 20 suprarenalectomized and to 13 control rats.

The lethal dose for the suprarenalectomized rats averaged 2.25 M.L.D. per gram of body weight as compared with 5.75 M.L.D. for the control rats, a ratio of 1 to 2.5. A constant post-mortem finding was a diffuse, edematous hemorrhagic condition of the lungs. The individual variation in the resistance of rats to diphtheria toxin is so marked that a determination of the exact lethal dose would require a large number of animals. Our results, therefore, are approximate and apply only to the particular strains tested by us. Nevertheless, they show a definite difference in the resistance of suprarenalectomized and normal rats to diphtheria toxin.

Disappearance of diphtheria toxin from the blood. The difference in resistance to diphtheria toxin in suprarenalectomized and normal rats suggests that in the former there is either an increased absorption or a decreased destruction or elimination of the toxin. An attempt was made to determine which of these factors predominated by measuring the amount of diphtheria toxin in the blood four hours after injection.

Methods. The toxin was administered intraperitoneally in doses equivalent to one M.L.D. per gram of body weight. Four hours after injection the rats were bled by cardiac puncture, the blood was immediately defibrinated, and the serum separated. Five dilutions of the serum up to 1:32 were made with physiologic saline. Intradermal injections of 0.1 cc. of each dilution were made by the Roemer method on the shaved abdomens of guinea pigs. Large, light colored guinea pigs were used because of the ease of reading and because Coca, Russell and Baughman have shown that consistent results can be obtained only with pigs weighing over 400 grams. Readings were made on the first, second and third days after injection. Glenny and Allen (1921) have observed that the intradermal reactions of diphtheria toxin are most sharply defined at the end of 36 hours.

The serum of a suprarenalectomized rat and of a control rat was tested in the same guinea pig in order to obtain a relative comparison and to

eliminate the possible error arising from the variation in the sensitiveness of different guinea pigs to diphtheria toxin. In addition each guinea pig received similar injections of diluted diphtheria toxin in order to determine its sensitiveness. The serum of each suprarenalectomized rat was tested in two or three pigs against different control sera. The cutaneous sensitiveness of guinea pigs to uninactivated normal and suprarenalectomized rat serum was found to be a negligible factor for the dilutions used in this test.

Results. Figure 1 represents graphically the comparative amount of diphtheria toxin in the serum of nineteen suprarenalectomized and twenty-

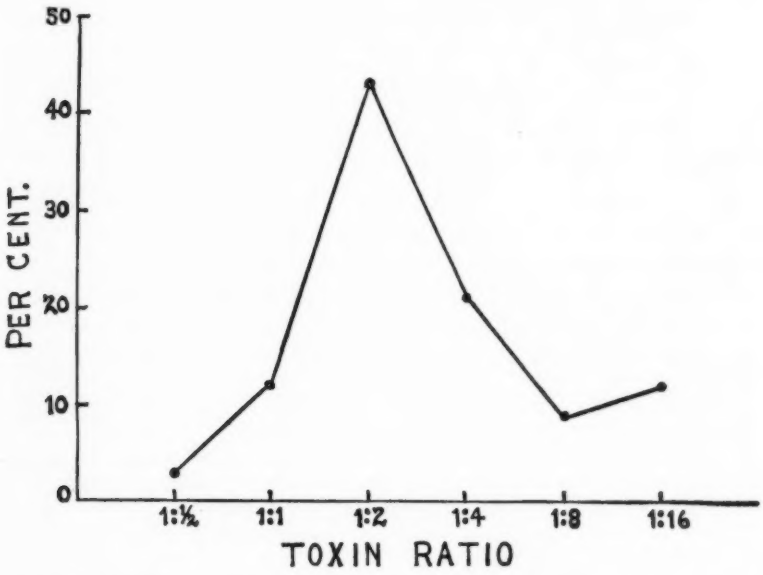

Fig. 1. Graph showing the ratio of the amount of diphtheria toxin in the blood serum of control and suprarenalectomized rats. The abscissae represent the ratios with the amount of toxin in the control rats as unity. The ordinates represent the per cent of tests giving the various ratios.

two control rats tested in thirty-three guinea pigs. For each guinea pig the comparative strength of the control to the suprarenalectomized rat serum was expressed as a ratio with the control as unity. The percentages of the different ratios constitute the curve, the mean of which falls between 1:2.5 and 1:3, or approximately at 1:2.75. In only one instance was the toxin content of the suprarenalectomized rat serum less than that of the control. The serum of the suprarenalectomized rats contained an average

of 1.36 M.L.D. of diphtheria toxin per cubic centimeter which is 2.75 times as great as the toxin content, 0.495 M.L.D. per cubic centimeter of the serum of the control rats.

Figure 2 represents the percentage of positive diphtheria toxin reactions for each dilution of serum for the two groups of rats. The actual curves are determined by the strength of the toxin in the rat sera and the sensitiveness of the various guinea pigs; but since one rat from each group was tested in the same guinea pig, a true difference is represented by these curves. Covering the period of maximum fall in each curve, average

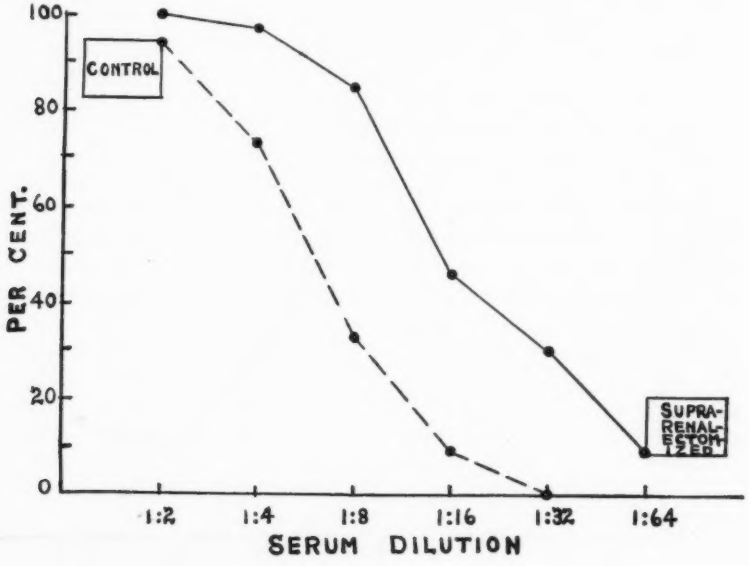

Fig. 2. Comparison of control and suprarenalectomized rats in respect to the amount of diphtheria toxin in the blood serum, as indicated by intradermal test in guinea pigs. The abscissae represent the serum dilutions. The ordinates represent the per cent of cases giving positive reactions for each dilution.

dilutions of 1:8 for the control rat serum and 1:24 for the suprarenalectomized rat serum, a ratio of 1:3, were obtained. A comparison of the areas enclosed by each curve after the beginning of its fall gives a ratio of 1:2.9.

In the same manner four rats, each with one suprarenal gland removed, were compared with rats which had undergone double suprarenalectomy and with operated and unoperated controls. The blood of the double suprarenalectomized rats had approximately twice as much diphtheria

toxin as that of the single suprarenalectomized rats. Three of the single suprarenalectomized rats had the same amount of toxin as the controls and one had four times as much. No difference was noted between the normal and blank operated rats.

DISCUSSION. Our observations confirm those of others that rats are not absolutely immune to diphtheria toxin. The susceptibility of normal rats varies with size, age, individual peculiarities, physical condition, and care after injection. Suprarenal insufficiency lowers the normal threshold of tolerance in rats. The presence of a greater amount of toxin in the blood of the suprarenalectomized rats than in the normal rats four hours after injection, although determined at only one period of time, suggests that the natural resistance of the rat to diphtheria toxin depends upon some mechanism for the elimination or destruction of the toxin. The greater concentration of the diphtheria toxin in the blood of the more susceptible suprarenalectomized rats may have some influence upon cell permeability and the attachment of the toxin to the cell, but according to our data it seems to be merely secondary to some primary factor of natural resistance.

SUMMARY

Between two and three weeks after the removal of the suprarenal glands rats are approximately two and one-half times as susceptible to diphtheria toxin as normal rats. The suprarenalectomized rats four hours after receiving an intraperitoneal injection of diphtheria toxin equivalent to 1 M.L.D. per gram of body weight have 2.75 times as much toxin in the blood as normal rats. Suprarenal deficiency apparently renders less effective the normal mechanism of the rat for the elimination or destruction of diphtheria toxin.

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THE USE OF LIGHT FILTERS IN COLORIMETRY WITH A METHOD FOR THE ESTIMATION OF HEMOGLOBIN

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The use of light filters for scientific purposes was first described by Leonardo da Vinci as early as 1519. He found that certain colored glasses were valuable in the study of his paints (3). For a long time colored glasses were the only light filters known but they served a purpose in many scientific investigations of fundamental character. There is a limit to the variety of color in glass, and unfortunately it seems to be very difficult for glass makers to reproduce the color in different meltings with any approach to exactness, so that for most purposes the modern light filters, made of gelatine impregnated with dyes, find a more extensive use (6). The type of light filters which are of greatest value in colorimetry is the selective or monochromatic filters. These have a sharp absorption curve and transmit a selected region of the spectrum more or less narrow. The importance of their use in colorimetry is at once suggested by the fact that the law governing the relationship between concentration and depth of solution is only true for monochromatic light. Colorimetry is a measurement of light absorption, and if a degree of accuracy is to be attained, such measurement should be made on the rays of light most absorbed (5), (4), (7). In order to confine absorption measurements to a particular spectral region where the solution in question has its maximum absorption, a prism may be used. In this case a spectrophotometer is emp oyed. Selective light filters when used with a colorimeter approach the same end.

Colorimetry applied to the analytical problems in biochemistry is subject to a serious limitation. The instability of biological material prevents its subsequent use as a comparison color standard. In spite of this limitation colorimetry remains the method of choice, and the reference standards employed include all manner of dyes, colored glass and inorganic salts. These simulate to a limited extent the color of the substance investigated, but the majority of such investigations leaves much to be desired as the colors are never identical and an accurate match in a colorimeter is often very difficult.

The present investigation was undertaken to determine to what extent

light filters might serve in the event of unsatisfactory comparison standards. The analytical method to be described was applied to blood solutions because of the convenient source of a pure biological pigment and because the results could be compared with a recognized quantitative method for hemoglobin, namely, the oxygen capacity method of Van Slyke (8).

It is well known that a solution of oxy-hemoglobin as well as the carbon-monoxide derivative has absorption bands with a maximum of about 540 $\mu\mu$. In choosing a color filter which would have a narrow transmission in this region, I selected the Wratten filter number 74, made by the Eastman Kodak Company. Figure 1 shows the absorption curves of this filter, oxy-hemoglobin and CO-hemoglobin plotted together. If the filter be placed in the eyepiece of a colorimeter the field will be illuminated by a fairly homogeneous green light. It is immaterial where in the path of light the filter be placed, so long as both fields of the instrument are affected equally. If a solution of hemoglobin be placed in the colorimeter cup on one side, the effect in the comparison field is absorption of green light; i.e., half the field appears dark green. The degree of this absorption can be altered by a change in the depth of solution. The problem now is to determine quantitatively the degree of absorption exerted by the solution at a given thickness. The Wratten neutral grey filters made by the Eastman Kodak Company are standardized for absorption for any wave-length, and these filters serve admirably for this work. If such a filter be placed in the colorimeter opposite to the blood solution, then a match in the comparison field is possible. At the match point the absorption by the solution and the filter is the same and is a known amount. The concentration of a solution is proportional to the amount of light absorbed and the thickness; or the ratio (called the absorption ratio) is a constant. The value of this constant is fixed for a given wave-length of light and for a particular substance. The absorption ratio for hemoglobin is determined experimentally, and with this value the concentration of unknown blood solutions may be readily found without the customary standards for comparison.

In reality the problem is more than colorimetry for it involves the principles of spectrophotometry.[1] Colorimetry usually implies a system of color specification, whereas the common analytical procedure in estimating concentration by a comparison method has nothing to do with color but correlates only the absorption of light rays by the solution and the absorption of the same rays by a standard. This standard may be a normal solution of the same substance, or if homogeneous light is used any other

[1] An effort to eliminate the present confusion in the nomenclature of the science of colorimetry has been initiated by the Optical Society of America and a preliminary report made. U. S. Bureau of Standards Library, Washington (D. C.) 1919.

device of photometry may be employed. The use of light filters and the adoption of principles of photometry become important in biochemistry, first, where stable standards are not available, as in the case of colorimetric hemoglobin estimations; secondly, where artificial standards are "off color" as illustrated by the method for bile pigment estimation using for comparison a solution of copper sulphate and potassium dichromate; thirdly, where it may be desirable to record concentration in absolute terms of light absorption, especially when the pigment has not been isolated in a pure state.

In connection with the present problem of using filters for monochromatic light in colorimetry it is remarkable that in 1852 A. Beer published the results of some experiments entitled "Bestimmung der Absorption des rothen Lichtes in farbigen Flüssigkeiten" (1). The necessity of using homogeneous light might be inferred from this title, and curiously enough homogeneous light was attained by using a light filter of red glass. The colorimeter used was of the polarizing type. Beer necessarily confined his observation to blue solutions on account of the limitation of the light filter used, but at the same time he wished that all regions of the spectrum might likewise be examined. It is only because of the light filters now available that this classical experiment can be extended.

Theoretical considerations. The validity of all colorimetric determinations is dependent on the fact that equal parts of homogeneous rays of light are absorbed by layers of equal thickness in a homogeneous medium, which may be expressed as

$$I' = I \cdot k^h \tag{1}$$

where I is the intensity of incident light, I' the intensity after passing through a layer of solution of thickness h, and where k is the transmission coefficient which multiplied by the value of I will give the intensity of light after passing through a solution of unit thickness. The value of k depends on the nature of the substance and also the wave-length of light. This obviously associates absorptive power with the number of molecules in the absorbing medium, or

$$I' = I \cdot k^{hc} \tag{2}$$

which is Beer's law; or, the absorption of light by a solution depends on the thickness of layer h, and on the molecular concentration c in the layer.

To simplify calculating concentration from the amount of light absorbed Bunsen and Roscoe (2) made use of what they called the extinction coefficient, e. It was defined by them as the reciprocal of that thickness which would reduce, by absorption, the intensity of incident light to one-tenth. Obviously, the value for the thickness which will absorb that

amount will be smaller, the greater the concentration. So by definition the extinction coefficient, e, and concentration, c, are proportional or

$$c : e = c' : e' \tag{3}$$

$$c/e = c'/e' = A \tag{4}$$

That is, the ratio of concentration to extinction coefficient is a constant, A, which is called the "absorption ratio." All spectroscopic absorption determinations depend on the evaluation of A. If the extinction coefficient, e, of a solution is determined optically and the concentration is known, then A is fixed for a particular spectral region. The concentration of an unknown solution can then be readily determined by finding the value of e optically and calculating from:

$$c = A \cdot e \tag{5}$$

For the evaluation of e, we have from equation (1)

$$I' = I \cdot k^h$$

Let $I = 1.0$, then $I' = k^h$

$$\text{Log } I' = h \cdot \log k$$

By definition of e, $h = 1/e$ when $I' = \frac{1}{10}$ and substituting above.

$$\text{Log } I' = 1 = \frac{\log k}{e}$$

Then

$$e = -\log k$$

$$= \frac{\log I'}{h}$$

or at unit thickness (1 cm.) the extinction coefficient is the negative log of the transmitted light. Now optical density, D, is defined as the negative log of the transmitted light, so,

$$\text{e} = \frac{D}{h} \text{ and if } h = 1 \text{ then e} = D \tag{7}$$

From (5)

$$c = A \cdot \frac{D}{h} \tag{8}$$

EXPERIMENTAL. *The determination of the absorption ratio A for blood solutions colorimetrically with light filters.* An ordinary laboratory colorim-

eter of the DuBoscq type was illuminated by a bright light source. Sunlight through a frosted window or the light from a 100 watt lamp was quite satisfactory. It was necessary to have an intense light source because the color filters used absorb 90 per cent and more of the total light. The plane mirror of the colorimeter was used instead of the frosted surface as a reflector. The Wratten filter number 74 was placed in the eyepiece, and the colorimeter adjusted so that both comparison fields were equal in intensity. A neutral grey filter of known density was placed on the cup holder on one side of the instrument. In this experiment the density of the filter used was 1.0 for the wave-length transmitted by color filter number 74.

TABLE 1

Oxygen capacity determinations of hemoglobin with average error and determination of constant A with deviations

SAMPLE NUMBER	HEMOGLOBIN PER 100 CC. BLOOD (BY O_2 CAPACITY) C_{obs}	MAXIMUM DEVIATION IN HEMOGLOBIN ±	COLORIMETRIC READINGS h	$\frac{D=1.00}{h}$ $=e$	C/e $=A$	CONCENTRATION CALCULATED $e\times14.8$ $=C_{cal}$	DIFFERENCE BETWEEN C_{cal} AND C_{obs}	BLOOD DILUTION IN COLORIMETER
	grams	*gram*	*cm.*			*grams*	*grams*	*per cent*
19	17.8	0.13	0.81	1.235	14.4	18.2	0.4	1
18	17.67	0.08	0.88	1.136	15.5	16.8	−0.7	1
17	17.0	0.20	0.82	1.220	14.5	18.0	1.0	1
16	18.85	0.22	0.81	1.235	15.2	18.3	−0.5	1
14	19.1	0.14	0.82	1.22	15.6*	18.0	−1.1	1
9	15.5	0.20	0.97	1.030	15.0	15.2	−0.3	1
8	18.8	0.10	0.83	1.205	15.6	17.8	−1.0	1
7	18.4	0.14	0.82	1.220	15.1	18.0	−0.4	1
15	16.0	0.11	0.93	1.075	14.9	15.9	−0.1	1
13	16.45	0.10	0.89	1.120	14.7	16.6	0.1	1
10	7.5	0.15	0.94	1.06	14.2	7.3	−0.2	2
6	7.4	0.12	0.97	1.03	14.4	7.2	−0.2	2
					14.8 mean value for A			

* Maximum deviation from mean for A = 5.4 per cent.
Average deviation from mean for A = 2.9 per cent.

From blood samples previously analyzed for hemoglobin content, 2 cc. portions were dissolved in 200 cc. of weak ammonia (1 cc. strong ammonia to 1 liter water). Illuminating gas was bubbled through the solution to saturation to effect clearing. It is to be noted here also that the transmission of the filter is nearer the maximum of CO-hemoglobin than oxyhemoglobin. See figure 1. The blood solution was placed in the colorimeter and a match attained with the density filter by adjusting the depth. Record was made of colorimetric readings in centimeters. Successive settings varied not more than 1 to 2 millimeters. Results are tabulated in table 1, column 4. Hemoglobin in grams per 100 cc. of blood may be

calculated from equation (8) by dividing the density of filter used (in this case density = 1.00) by the colorimetric readings in centimeters and multiplying by a constant A, which was determined in this experiment to be 14.8 ± 0.4. This constant is referred to as the absorption ratio. In column 7, table 1, the concentration of hemoglobin is recalculated on the basis of the extinction coefficient, e, (as found from colorimetric readings) and the average value for A or 14.8. See equation (5).

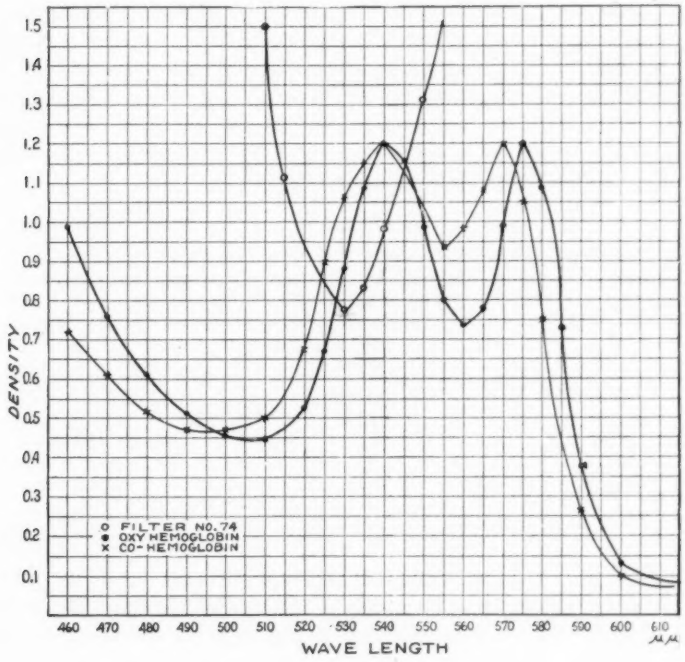

Fig. 1

The determination of concentration of hemoglobin by oxygen capacity. The concentration of hemoglobin used in the preceding determination was measured by the oxygen capacity of blood (8). Dog's blood was taken from normal animals and also those with experimental anemia (9). The venous blood as drawn was either citrated or defibrinated and then subjected to complete aeration. On 2 cc. portions of each sample the combined oxygen was determined by the method of Van Slyke. At least three repeated determinations were made on each of two types of apparatus; one measured the oxygen at constant volume and the other measured the

gas at constant pressure. From the data thus obtained the average volume of O_2 for 100 cc. of blood was calculated and the equivalent in grams hemoglobin determined by using the factor 0.746. See table 1, column 2.

A spectrophotometric check on the value of A. The mean transmission of filter number 74 is assumed to be between 520 $\mu\mu$ and 540 $\mu\mu$ which is apparent from figure 1. In a given solution of blood of known concentration, the densities at wave-lengths between 520 to 540$\mu\mu$ were determined on a spectrophotometer and a value for A at each wave-length was calculated. As an experiment, a sample of dog's blood was analyzed for hemoglobin by the oxygen capacity method and found to contain 17.0 grams per 100 cc. A 1 per cent solution was made, saturated with CO and examined in a Bausch & Lomb spectrophotometer with a cell 1 cm. thick. The constant A was calculated from the density determined optically. The average value of A between 520 to 540 $\mu\mu$ was 14.8 or the same as that obtained with the colorimeter and filter number 74. See table 2.

TABLE 2

Values of A determined spectrophotometrically between 520-540$\mu\mu$

Blood sample contained 17 grams hemoglobin per 100 cc. 1 per cent CO-hemoglobin solution in 1 cm. cell.

WAVE LENGTH	DENSITY D	$\frac{17.0\ g}{D} = A$
520	0.830	20.5
525	1.020	16.6
530	1.250	13.6
535	1.415	12.0
540	1.480	11.5
		14.8 mean value for A

Discussion. A factor, called the absorption ratio A has been determined for CO hemoglobin, by means of certain light filters. The average value is 14.8. For the determination of hemoglobin in unknown solutions, one proceeds in the same manner as outlined for the determination of the factor. The blood solution is converted into the CO derivative and read in a colorimeter, with two filters, one a color filter and the other a filter standardized for density. By dividing the density value of the latter by the colorimetric reading and multiplying by the above factor, a value for concentration is obtained in grams hemoglobin per 100 cc. of blood.

It may be inferred from the results in table 1 that the concentration of hemoglobin in various samples of blood was determined by the method of Van Slyke within 1.5 per cent accuracy. The error of the colorimetric method described is about 3.0 per cent. The apparent wide deviations in experiments 8, 14, 17 and 18 may very well have been due to inaccuracy in securing a proper equalization of illumination on the colorimeter before

observations were made. The illustration used here in the analysis of hemoglobin is as difficult as that of any biological substance since the spectral absorption of hemoglobin is complicated by two sharp absorption bands. The method has been used for the investigation of other substances, such as bile and urochrome, and in addition some inorganic salts were analyzed with considerable facility and accuracy.

SUMMARY

1. A method for using light filters in colorimetry has been described.

2. As an illustration, analyses have been made on hemoglobin and a comparison made between the oxygen capacity method and the colorimetric method using light filters, showing an average deviation of 2.9 per cent.

3. Further comparison was made in the determination of the absorption ratio A by this colorimetric method and by a spectrophotometric method with close agreement.

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STUDIES ON THE VISCERAL NERVOUS SYSTEM

XVII. Reflexes from the Colon

1. reflexes to the stomach

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The basis for the modern study of gastro-intestinal reflexes was laid by Bayliss and Starling (1) in 1899 when they reported that handling the intestine with the fingers "produced reflex inhibition of the whole length of the intestine." This reflex was abolished by section of the extrinsic nerves but was not influenced by section of the gut. It was, therefore, a reflex via the extrinsic nerves alone. No attempt was made to show the application of this reflex to gastro-intestinal physiology.

In 1906 Cannon (2) stated that he was not only able to secure an inhibition of the stomach via the extrinsic nerves by stimulating the small intestine, but that he was able, also, to get such a reflex with the extrinsic nerves cut. He concluded that the reflex travelled mainly via the extrinsic nerves, but could also travel equally well via the intrinsic "enteric system."

Carlson (3) in 1914 corroborated Cannon by reporting that hunger contractions of the stomach could be inhibited via both extrinsic and intrinsic pathways when the intestinal mucosa was stimulated chemically or mechanically. Carlson first called attention to the possible significance of this reflex, as far as the hunger mechanism is concerned. The precise rôle of this reflex in the control of the gastric hunger mechanism in the normal animal must be determined by further investigation. It is probably a factor in the diminution or absence of hunger in cases of enteritis, intestinal obstruction and constipation.

Cannon (4) observed that section of the intestine 18 inches below the pylorus caused the pylorus to remain in spasm for five hours, although the stomach showed vigorous digestion peristalsis.

Neither Carlson nor Cannon state that they obtained reflex effects upon the stomach by stimulation of the gut at other than short distances from the stomach. There is, therefore, no real discrepancy between their results and those of Bayliss and Starling. The latter dealt with long

enteric reflexes and found the nervous pathway extrinsic, whereas the former dealt with short enteric reflexes and found extrinsic and intrinsic nervous pathways.

The clinical literature soon contained numerous instances of delayed emptying time of the stomach as a result of intestinal stimulation (5). No statement is made, however, as to whether the delay was due to inhibition of the stomach or to spasm of the pylorus.

EXPERIMENTAL METHODS. *A. To study the possibility of long enteric reflexes: Frogs and turtles.* The entire gastro-intestinal tract from the gullet to the cloaca was removed and placed in a shallow vessel containing Ringer's solution to the depth of from one-sixteenth to one-eighth of an inch. A piece of gauze laid over the gut served, by its wick-like action, to keep the gut well moistened and also to facilitate maximal aeration.

Record of the gastric motility was made by two methods, the balloon and the enterograph. The balloon was inserted through the gullet, or the stomach itself was fixed to serve as a balloon by tying a cannula into the cardiac end of the stomach and closing the pylorus with a delicate rubber band; the balloon, or the cannula, was connected to a small water manometer of 7 mm. inside diameter. When the enterograph method was used the pyloric end of the stomach was fastened by a suture through its wall on a metal rod. A silk thread from the cardiac end of the stomach was attached to a heart lever. Records were made with the stomach suspended or hanging from the fixed point or with the stomach lying on the bottom of the vessel, thus eliminating the influence of tension.

While a record was thus being made of the tonus and contractions of the stomach, the colon was stimulated. The types of stimulation were chosen with the view to duplicating those acting in the colon of the living animal, viz., distention, elongation (stretching), acids and bases, and protein split products. Electrical stimulation was also used.

The colon was distended in two ways: a tiny balloon was introduced through the cloacal opening, or a cannula was tied into the opening and a delicate rubber band was tied around the gut at the ileocolic juncture or at about an inch from the pylorus. When the latter method was used, air or carbon dioxide were used for distention. Various pressures were used; a low pressure for a long period, a gradually increasing pressure the maximum at times being sufficient to rupture the gut, a sudden maximal pressure, alternating distention and relaxation, etc. The colon was elongated gently between the fingers or between hemostats. As in the case of distention various degrees and rates of elongation were used.

Acids and bases singly or alternately, in concentrations varying from weak to quite strong, were injected into the colon through the cloacal opening. Histamine, choline chloride and cholamine were used similarly.

The galvanic, both continuous and interrupted, and the faradic currents, both very weak and very strong, were used. The electrodes were placed inside the lumen of the colon or inserted through its walls.

The series of experiments was repeated using the colon as the effector end and the stomach as receptor by reversing the apparatus with suitable minor modifications.

Dogs and cats. These animals were prepared by light ether anesthesia, light barbital hypnosis (6), and by decerebration by the Davis method (7) of ligation of the carotid arteries in the neck and the basilar artery just caudal to the circle of Willis. The decerebration method made very sensitive cat preparations, but was usually not satisfactory in the dog, because of the limited and variable decerebration which it produced.

The colon was stimulated by distention, by mechanical irritation, by acids, and by the electrical current. The electrical current was the first method of choice because its intensity can be regulated so easily and because it can be applied without the mechanical displacement of the viscus. It was subsequently discarded, however, because it was found that the escape of the current could not be controlled. Acid was applied with a swab introduced through a glass tube about four inches in length. This tube prevented acid from coming in contact with the rectal walls. Mechanical stimulation was performed with a test tube brush introduced through a similar glass tube in order that the rectal mucosa should not be stimulated.

In order to apply adequate distention it was found necessary to construct a special apparatus. It is a divulsor built upon the principle and suggested by the cardiaspasm-divulsor of Sippy. Around a small brass tube containing several holes toward one end, a rubber condom is secured by a cord tying the condom to the tube. A thin silk bag about two and one-half inches in diameter is then similarly tied over the condom. A second condom is then fastened over the silk bag. We now have a silk bag with a condom within and one outside of it. The bag is distended by the inner condom when air is pumped into it through the metal tube; the bag permits several hundred millimeters of mercury pressure within the balloon. The outer condom keeps the bag clean and dry.

The record of the gastric motility was taken by the balloon and the enterograph method. The regular balloon method was used, the balloon being introduced through an opening in the cervical esophagus.

The one-wire-fixed-point enterograph method was used. The lesser curvature of the stomach near the incisura angularis was chosen for the fixed point. The moving point was on the greater curvature opposite the fixed point. A silk thread is attached to the moving point selected and passed out from the abdominal cavity through a glass tube placed in its walls. The abdominal walls are pulled away from the stomach

and held there so that there is a large tent-like space allowing free motility of the stomach without being influenced by its neighboring tissues. The thread, after leaving the abdominal cavity, passes over pulleys to a heart lever. A weight attached to the heart lever keeps the system taut and the stomach in proper tension.

B. To study the gastric response over the extrinsic nerves. Dogs and cats were used and were prepared as described earlier in the paper. Stimulation of the colon and records from the stomach were performed as described above.

In order to assure ourselves that our stomach records were purely from the stomach and not influenced by the adjacent structures, we placed a large rubber balloon between the abdominal wall and the stomach. By using this intra-abdominal pressure record as a control for the stomach, we were able to ascertain clearly that the tonus and motility of the stomach were faithfully recorded by its own recording mechanisms, and that this record was quite uncomplicated by intra-abdominal pressure changes due to the contraction of the abdominal musculature or the diaphragm. Experiments upon completely curarized animals gave identical results.

The effects of colon stimulation upon the motility and tonus of the stomach during digestion were studied in normal anesthetized dogs, in lightly anesthetized normal dogs, and in decerebrate dogs. Some observations were also made on the empty stomach of unanesthetized dogs.

RESULTS. *A. Colon to stomach.* In all cases adequate distention of the colon produced a diminution or cessation of contractions and variable fall in tonus of the digesting or empty stomach. The degree of inhibition depended upon the condition of the animal and the intensity and duration of the stimulation of the colon.

Figure 1 was obtained by the balloon method from a normal conscious dog. It shows the effect of distention of the colon upon the hunger contractions of the stomach. The insertion of the colon distending balloon at *a* produced immediate and marked inhibition of the stomach lasting somewhat more than one minute. Distending the colon balloon produced an immediate and very marked fall of tonus and complete inhibition of the stomach, lasting for the period of the stimulation. Very shortly after the release from the first inhibition, the tonus sharply rose. The first hunger contraction which followed immediately the sharp tonus rise, reached a height of one inch of water pressure above the prestimulation limit, and one and a quarter inches above the succeeding contractions. Following the second inhibition, the tonus and motility responded similarly by a "rebound" with the differences, however, that the "rebound" contraction was not so high and that the rhythm apparently changed abruptly. Close inspection, however, will reveal the fact that the rhythm of the hunger contractions is unchanged and that the slow rhythm is a

tonus rhythm. The tonus rhythm is quite striking. The peaks are higher than those of the hunger contractions just preceding the second inhibition period, whereas the tonus was so low at the bottom of the waves that rather deep respiratory excursions are recorded. It is interesting to note further that the degree of "rebound" is not related to the length of inhibition, as in the second case the rebound is smaller than in the first case, where the inhibition was of shorter duration.

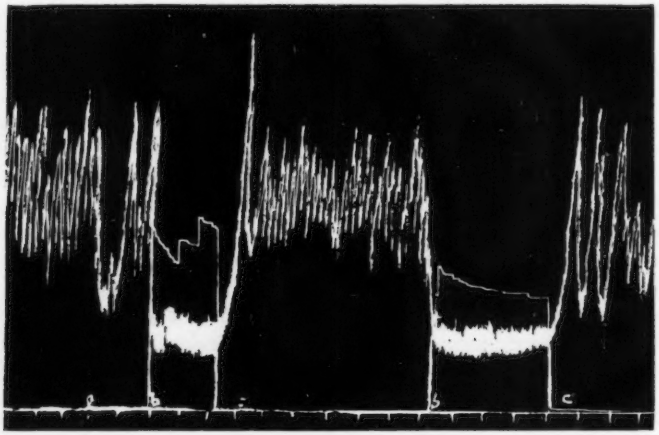

Fig. 1. Balloon-water-manometer method record of hunger contractions of a normal conscious dog. Stimulating balloon was introduced into colon at *a*, causing immediate and marked inhibition of the stomach. The colon balloon was distended with 100 mm. Hg and 80 mm. Hg between the two periods *b–c*.

Note the abrupt and very marked fall in tonus and the complete inhibition of hunger contractions during the stimulation intervals. Note also the prompt release from inhibition after the cessation of the stimulation and the "rebound" following each inhibition period, and the altered rhythm following the second inhibition period.

Upper record is water manometer record from stomach balloon. The record of the stimulating colon balloon is in millimeters Hg. The bottom line is the base line for both records. Time interval, one minute. The record is reduced one-half.

In a few experiments, especially in those recorded by the enterograph method, the rhythmic response to stimulation was considerably different from that described above. The tonus drop was somewhat less than that usually obtained. The digestion peristalses, however, ceased immediately and completely. After the cessation of the colon distention, the contractions did not return with a brisk "rebound," but, on the contrary, gradually increased in amplitude, and even in rate, until the normal was reached. The prestimulation rhythm was 10 seconds. After the cessa-

tion of stimulation, it was 23 seconds, 15 seconds, 13 seconds, 12 seconds, 11.5 seconds and 11 seconds, respectively, for the first seven waves.

In suitable barbitalized preparations, complete gastric inhibition could be obtained by 20 mm. to 40 mm. Hg colon distention. After a period of from one-half to one minute, the contractions "broke through" the inhibition. An additional distention of 5 mm. or 10 mm. Hg again produced a complete inhibition, which after a short period "broke through." This could be repeated regularly until sufficient distention was obtained to maintain complete inhibition.

Hunger contractions in normal, conscious dogs could be inhibited with ease. A colon distention of 20 to 40 mm. Hg for ten to fifteen minutes was sufficient to produce an inhibition of the stomach, from which it would not reach its prestimulation normal for several hours. The "recovery phase" was constant and gradual. Quiescent hunger phases did not complicate the interpretion of the records.

In one dog under light ether anesthesia, distention of the colon produced a gradual rise of tonus which within five minutes increased by two

Fig. 2. Balloon-water-manometer method record of jejunum of dog under barbital narcosis. Colon distended at *a* and pressure released at *b*. Note marked inhibition of tonus and cessation of contractions. Also note marked "rebound" after inhibition periods.

inches of water pressure. A slow rhythmical wave of considerable intensity superimposed itself upon it. The stomach was previously quiescent and had been so for an hour.

The small intestine. In figure 2 is seen marked inhibition of the small intestine from colon stimulation in a barbitalized dog. Similar responses were obtained from the esophagus, stomach, jejunum and ileum. We wish to point out the rebound response following release from the inhibition, exactly similar to that observed in the stomach records.

Stimulation of the stomach, small intestine, or colon inhibited the esophagus. The motility and tonus of the colon were inhibited by stimulation of the esophagus, stomach and small intestine.

The sphincters. Distention of the colon or small intestine produced moderate inhibition of the cardiac and pyloric sphincters. The ileocolic sphincter, on the contrary, responded by marked contraction.

B. Over the intrinsic nervous mechanism. 1. On the isolated gut of frogs and turtles. In no case did any type of stimulation of the colon alter the tonus or motility of the stomach. In the reverse series of experiments,

no type of stimulation of the stomach altered the tonus or motility of the colon. The series was repeated using the gut of strychninized frogs and applying the various stimuli even high up the small intestine. The results were again uniformly negative. To eliminate the possible "shock" effects of removal of the gut from the body, similar experiments were performed upon the "pithed" and "decerebrate" bullfrog, Rana catesbiana, in which the gut was used with its blood supply intact. The results were the same as in the other series—that is, completely negative. We were unable to influence the tonus or contraction of the stomach by stimulation of the large or small intestine and we were unable to influence the motility of the colon by stimulation of the stomach.

2. In the dog and cat, with intact cord and visceral nerves, it was found that colon stimulation caused inhibition of the tonus and motility of the stomach. Section of the extrinsic nerves to the colon, section of the spinal cord, or section of the splanchnics to the stomach caused the abolition of this inhibition reflex. Furthermore if the extrinsic nerves to the colon and stomach were intact, section of the small intestine did not alter or modify the reflex. As in the case of frogs and turtles, we were unable to influence the tonus or contractions of the stomach by stimulation of the large intestine—in the absence of the extrinsic nerves.

Discussion. Previous investigators obtained inhibition of various portions of the gastrointestinal tract by stimulation of certain portions. We believe that the present evidence justifies the conclusion, at least as a working hypothesis, that adequate distention of any portion of the gastrointestinal tract will produce inhibition of every other portion of the gastrointestinal tract, with the exception of the ileocolic sphincter.

Our studies upon the mechanism of this reflex are not yet complete. All of our evidence, however, indicates that it is not a matter of gradient. The gradient theory demands an altered motility, such as a backward activity (8). We have observed all gradations of inhibition of the stomach and intestines to practically complete inhibition but anti-peristalsis never occurred. In fact it is very difficult to imagine motility of the gut in either direction at the marked degrees of inhibition which we observed in as much as motility was suspended, apparently, *because* of the fall in tonus.

In analyzing these inhibition responses one is struck by the similarity which they bear to those obtained by vagal inhibition of the heart. In both cases with stimuli of sufficient intensity there is a marked fall in tonus with cessation of contractions, in both cases under suitable conditions the contractions "break through" the inhibition, in both cases resumption of motility is inaugurated by increased activity or "rebound." The gastric inhibition seems to be primarily dependent upon a rapid fall

in tonus, the degree of inhibition of contractions and the degree of hypermotility being directly proportional to the degree of tonus.

It is interesting to note that the small intestine responds in exactly the same manner as the stomach. We thus corroborate and extend the work of King (9).

The studies on the nervous mechanism involved in these reflexes have not been completed, but it is suggestive that every response obtained is exactly what one would expect if the efferent arc were over the sympathetics. Although, as has been demonstrated, both the vagi and the sympathetics are both inhibitory and motor under appropriate circumstances (10),(11) the sympathetics are predominantly inhibitory except to the ileocolic sphincter where they are solely motor (12). If the reflex responses utilize the sympathetic efferent pathways one would expect inhibition of the cardiac and pyloric sphincters and contractions of the ileocolic, and inhibition of the stomach and small intestines. The results are in accord with the demands. Furthermore section of the vagi did not interfere with these reflexes.

The fact that the inhibition response occurred so soon after the stimulation eliminates the possibility of epinephrin playing a principal rôle. The response occurred within one to two seconds after stimulation, far too quickly for an adrenal product to reach the stomach via the blood stream.

The most obvious possible source of error in the experiments on the isolated gut of frogs and turtles is of course the "shock" factor. We do not believe that such error operated here, *a*, because we used animals phylogenetically and ontogenetically young and therefore least susceptible to "shock," and *b*, because the stomach and colon of these preparations continued to contract rhythmically and regularly for hours. Furthermore, we used several Rana catesbiana that had been decerebrated and fistulas made into the stomach and the colon several days prior to the experiments and which were in excellent condition. These animals were certainly not in "shock." These latter experiments also eliminate the possible error due to asphyxia, for the blood supply to the gut was not interfered with.

It is interesting to note further that in these decerebrate Rana catesbiana preparations no "gastro-colic" or colon gastric reflexes were obtained even though the complete nervous system was intact (except for the decerebration). A "gastro-colic reflex" has frequently been observed in man (13). It has more recently been observed in the dog by Ivy[1] (14).

[1] Ivy found that this is really a "duodeno colon reflex" at least in the dog, for stimulation of the duodenum and upper jejunum caused defecation whereas distention of the stomach never had such an effect (15).

Therefore having failed to obtain either of these reflexes in frogs and turtles, and having obtained both, in dog and man, it seems reasonable to conclude that these reflexes are not primitive, but are of recent origin. Thus one would expect that such reflexes would utilize the newer, more highly specialized reflex pathways.

SUMMARY

1. Distention of the esophagus, duodenum, jejunum or colon, in dogs and cats, produced immediate inhibition of the empty or digesting stomach as manifested by sharp and marked fall in tonus and cessation of contractions (the inhibition lasting as long as sufficient distention was maintained) and produced inhibition of the cardiac and pyloric sphincters and contractions of the ileocolic sphincter.

2. Distention of the esophagus, stomach, duodenum, ileum, or colon produced similar responses of the jejunum, esophagus and colon.

3. The inhibition phase is followed by a positive "rebound" phase, suggestive of that obtained after vagal stimulation of the heart. The inhibition appears to be fundamentally due to a rapid fall in tonus, the rate and amplitude of contractions varying proportionally with the degree of tonus.

4. These inhibitions of the stomach and intestine travel presumably over the sympathetic efferent nerves and are not primarily due to any adrenal product as the latent period is far less than the necessary circulation time.

5. Distention of any portion of the gastrointestinal tract will produce inhibition of every other portion of the tract; the part stimulated will respond by contraction.

6. In frogs and turtles it was found impossible to obtain long reflexes over the gut in either an orad or a caudad direction via the mechanisms resident in the walls of the gut, and in dogs and cats such reflexes could not be obtained via these mechanisms, at least in an orad direction.

7. It is concluded, as a working hypothesis, that reflexes involving phylogenetically old mechanisms in the gut act locally; that reflexes acting in the gut at a distance from the stimuli, are phylogenetically recent and involve recent nervous structures.

We acknowledge with pleasure our indebtedness to Doctor Carlson for his constant coöperation and criticism.

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THE EFFECT OF SUPRARENIN AND THYROXIN ON WATER ABSORPTION BY BRAIN TISSUE

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A number of investigations indicate that the secretions of the suprarenals and thyroid affect the physico-chemical condition of protoplasm. Beaman (1905) has called attention to the swelling and localized areas of edema which occur among the unfavorable clinical results that sometimes accompany the use of adrenalin solutions. He has demonstrated by different types of experiments a direct action of adrenalin on protoplasm. Thus, when active spermatozoa of the sea-urchin are introduced into a solution of sea-water and adrenalin (0.0001), they soon lose their motility and within two hours are dead. If, however, they are brought into a solution of much weaker strength (0.000013), their motion is immediately checked, but is recovered again in fifteen minutes, and their normal activity resumed. In like manner, the cilia of the sea-urchin blastula are slowed and stopped in their movement when placed in an adrenalin solution of 0.0001. Adrenalin, it would seem, therefore, has a direct detrimental action on the protoplasmic activities of these bodies. On the other hand, it serves as a stimulant to the protoplasm of muscle. An excised turtle heart that has ceased to beat is restored when bathed in an adrenalin solution.

Meltzer (1905) observed that suprarenal extract retards absorption from the tissues into the blood and transudation from the blood into the tissues. This observation he explains by assuming that the extract increases the tonicity of the protoplasm surrounding the pores of the endothelia in the capillaries.

An histological study by Drummond (1904), after an injection of adrenalin, demonstrates its toxic effect on the protoplasm of the internal organs. In addition to the extravasation of blood and congestion which were sometimes observed, Drummond describes marked changes in the kidneys which varied from congestion of the organ with cloudy swelling of the epithelial cells to a parenchymatous nephritis and a desquamation of cells in the tubules. Some animals that were injected with the glandular

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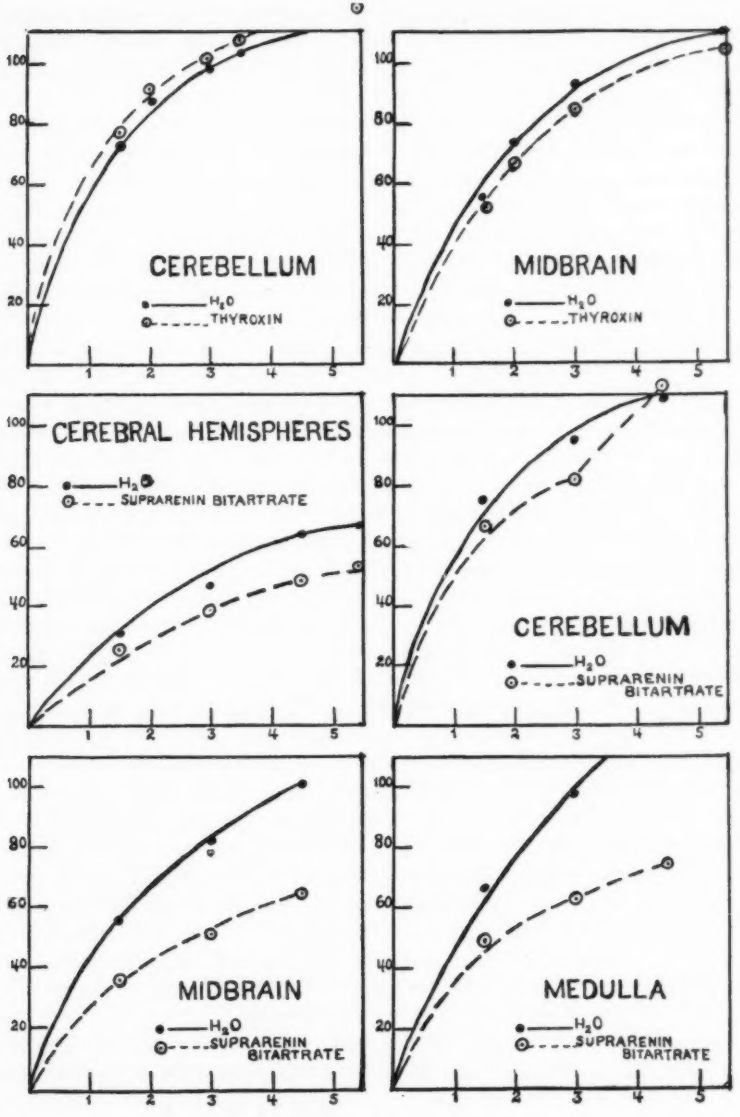
CEREBELLUM
H₂O
THYROXIN
MIDBRAIN
H₂O
THYROXIN
CEREBRAL HEMISPHERES
H₂O
SUPRARENIN BITARTRATE
CEREBELLUM
H₂O
SUPRARENIN BITARTRATE
MIDBRAIN
H₂O
SUPRARENIN BITARTRATE
MEDULLA
H₂O
SUPRARENIN BITARTRATE
ABSCISSA = HRS.
ORDINATE = % INCREASE

extract died in convulsions resembling asphyxia. The cause of death in most instances was edema of the lungs. Two factors, it seems, were involved: 1, the influence of adrenalin upon blood pressure; 2, a direct toxic action. The inflammatory changes were due to the latter.

Since the protoplasm of brain cells which is colloidal in nature manifests the same colloidal behavior as the protoplasm of the other organs (Fischer, 1921), one might therefore expect suprarenin and thyroxin to exert a specific effect on the cells of the central nervous tissue. That such is the case is evidenced by studies which indicate a direct action of adrenalin on the respiratory centre. The view of direct action was held by Boruttau (1899) and likewise by Garrelon (1912). More recent evidence has been produced by Huggett and Mellanby (1924).

As to the specific effect of the thyroid principle on protoplasm, Schäfer (1916), (1922) advances the probable opinion that the tremors of the muscles and the psychical excitement of hypothyroidism are the results of the direct action of the thyroid hormone on the central nervous system. Burridge (1923) believes that the augmentor influence of an aqueous extract of the thyroid on the heart beat acts by producing in the colloids of the cardiac excitable structure a finer state of subdivision than normal, ipso facto conferring on the heart an increased capacity for function. This view, in the opinion of the writers, is highly suggestive but by no means conclusive. The recent report by Kunde (1926) that in thyroid administration (either the desiccated by mouth or Kendall's thyroxin injected intravenously) to hypothyroid animals, water is extracted from the tissues, indicates a direct action on protoplasmic colloids. This view finds confirmation in the conclusions of Danzer (1926) which he draws from Eppinger's (1917) experimental studies and his own clinical observations that the thyroid principle regulates the flow of water to and from the tissues.

THE PROBLEM. The evidence adduced for a direct effect of adrenalin and the thyroid extract on protoplasm suggested the present problem. It has been shown (Haldi and Rauth, 1926) that the four parts of the rabbit's brain—cerebral hemispheres, midbrain, cerebellum and medulla—give different types of water absorption curve. This fact may be interpreted as an indication of colloidal differences (due to chemical constitution) of the brain substance. Do the secretions of the adrenals and the thyroid gland affect the hydration of the brain cells? An attempt to throw light on this question is embodied in these experiments.

EXPERIMENTAL METHODS. A rabbit was taken quietly from its cage and decapitated. The dura was removed and the brain taken out of the skull box and divided into four parts—the cerebral hemispheres, cerebellum, midbrain and medulla. The midbrain was separated from the hemispheres by clipping with a sharp scissors close to the latter. Each of the four parts was then divided into symmetrical halves and dropped into a

weighing bottle, one into the solution under study, and its control into distilled water. Each bottle contained 15 cc. of liquid.

The time interval between decapitation and immersion of the brain was kept constant. In order to safeguard against bacterial decomposition, the operation was conducted under sterile conditions. Previous observations in these laboratories having shown that temperature affects the rate of water absorption, a temperature practically constant was maintained in these experiments. Immediately after the brain portions were put into the solution, the bottles were weighed, and the weight of the brain determined by subtracting the weight of the bottle plus solution (previously determined) from the weight of the bottle plus solution and brain portion. The bottles were then placed in an oven the temperature of which varied during the experiment from 35°C. to 37°C. At specific intervals the brain portions were placed on a watch crystal, weighed, and replaced in the weighing bottles.

The thyroxin of Kendall (Squibb's), suprarenin base, and suprarenin bitartrate (supplied by the H. A. Metz Laboratories) were used. Three methods were employed in the study of thyroxin effects: 1, thyroxin crystals were dissolved in one drop of 10 per cent NaOH and diluted to 100 cc. The control solution was prepared by diluting one drop of 10 per cent NaOH to 100 cc. with distilled water; 2, two drops of 10 per cent NaOH were diluted to 200 cc. with distilled water and 100 cc. used for the controls. Thyroxin crystals were dissolved in the remaining 100 cc. 3, Thyroxin crystals were gently heated in distilled water, shaken, and filtered. These precautions were taken against any error that might be introduced through the use of NaOH. Each type of experiment gave qualitatively identical results. No difficulty was experienced with the suprarenin bitartrate solution as it goes readily into solution in water. Fifty milligrams were dissolved in 100 cc. distilled water. Suprarenin base is much less soluble in water. Different amounts were shaken in a volumetric flask with distilled water and filtered. The filtrate undergoes a series of color changes which are due to an oxidation process. In our check experiments an attempt was made to maintain all time intervals constant.

Results. The accompanying graphs are plotted from our experimental data. The swelling of the cerebral hemispheres and the medulla in thyroxin solution was the same as the controls within the limits of experimental error. The cerebellum in the thyroxin solution swelled more and the midbrain less than their respective controls. Although the difference is not a marked one it was nevertheless constant for every weighing.

In the suprarenin bitartrate solution the four parts of the brain swelled less than in the distilled water. At the third weighing the water absorption curve of the cerebellum in thyroxin rises abruptly and continues gaining over its control.

Graphs are not constructed for the results with suprarenin base, as in a few of our experiments the results were reversed with the cerebellum and and in one instance with the hemispheres and medulla. This we believe was due to the oxidation of the base in solution which was difficult to control.

In most of the experiments the swelling of the hemispheres, cerebellum and medulla was greater and in every case that of the midbrain was less in the suprarenin base solution than in distilled water. Regardless of the amount of oxidation we conclude that the midbrain absorbs less water in the suprarenin base solution than in distilled water.

Discussion. The intimate relationship between mental life and the protoplasm of the central nervous system suggests a colloid chemical basis for mental disorders. The brain tissue is a colloid with a water content of 85 per cent in the gray matter of the adult human brain and 70 per cent in the white matter. We may assume a physico-chemical equilibrium existing between the brain cells and the blood and a diminution or increase of the hydration of the cells when the equilibrium is disturbed. The manifold character of psychic disorders finds a possible explanation in this concept, since it is reasonable to conceive a psychic correlate of the physico-chemical disturbance in the brain cells. Various portions of the brain substance have been shown (Haldi and Rauth, 1926) to manifest specific hydration capacities which are affected differently by the same chemical substance. This is probably true likewise of smaller groups of cells.

A disturbance in equilibrium might be induced by a number of factors, change in the calcium colloid-ionic ratio, the concentration of electrolytes, the hydrogen-ion concentration of the blood, etc. Since clinical observations point to a dysfunctioning of the endocrine glands in a number of mental disorders, it seems that a hyper- or hypo-secretion of the glands might disturb the brain tissue-blood equilibrium and thereby affect the hydration of the brain cells.

The objection may be raised to the type of experiments reported in the study, that we are not dealing with living protoplasm and that they therefore do not represent what actually occurs *in vivo*. This is unquestionably a serious objection. Furthermore when an animal is decapitated and the brain removed there is a rapid accumulation of lactic acid (McGinty and Gesell, 1926) so that another factor is introduced that is not found in the normal conditions of the living animal. However, Fischer's experiments (1921) show that at least in some physico-chemical relations there is not an essential difference between living and dead protoplasm. Moreover, the accumulation of lactic acid in the brain after decerebration (which may account for its increased water capacity) might occur in a lesser degree in

pathological conditions. Thus Liesegang (1910) associates the swelling of the brain in thymectomized dogs with a hyperacidity of the gray matter.

Since, therefore, the present experiments show a differential effect produced by the synthetic products, thyroxin and suprarenin, on water absorption by the brain cells, it is probable that *in vivo* any marked change in the functioning of the thyroid or adrenals affects differentially the hydration of the brain cells. This assumption is borne out by the experimental studies on other tissues referred to in the literature.

We do not wish to minimize the complexity of the two systems, brain protoplasm and the blood. From the colloid chemical point of view their elements may mutually enforce, or, on the other hand, counteract one another. An illustration of this condition is found in the studies of the different ions in colloidal systems (Höber, 1922). Our experiments therefore most probably do not give an exact representation of what occurs *in vivo*, but they do, however, suggest some important physico-chemical relations.

SUMMARY

1. A study of the effects of thyroxin, suprarenin bitartrate and suprarenin base on the water absorption by brain tissue is reported.

2. Water absorption by the cerebral hemispheres and medulla is the same in thyroxin solution as in distilled water.

3. The cerebellum swells more and the midbrain less in thyroxin solution than in distilled water.

4. The cerebral hemispheres, the cerebellum, the midbrain and medulla swell less in suprarenin bitartrate solution than in distilled water.

5. Suprarenin base dissolved in water oxidizes upon standing. Consequently a study of the effect of the base on water absorption is attended with difficulties. Swelling varies quantitatively and occasionally qualitatively with different degrees of oxidation.

6. The hemispheres, cerebellum and medulla generally swelled more in the oxidized solution of the base, and the midbrain invariably less than in distilled water.

7. A colloid chemical basis of mental disorders is discussed.

We wish to acknowledge our indebtedness to Dr. Carl Hezog of the H. A. Metz Laboratories, who kindly supplied us with a generous amount of suprarenin bitartrate and suprarenin base.

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THE INVOLUNTARY CONTRACTION FOLLOWING ISOMETRIC CONTRACTION OF SKELETAL MUSCLE IN MAN

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The "post-contraction" described by Salmon (1914) and Kohnstamm (1915) and called by the latter "catatonus," consists in a somewhat prolonged involuntary contraction of a skeletal muscle following the cessation of a powerful, voluntary, isometric contraction lasting for five seconds or more. It is best induced by an isometric contraction lasting at least twenty seconds. The phenomenon was brought to the attention of one of us in a manner which practically eliminated the possibility of mental suggestion as the cause. The subject was instructed to make the isometric contraction and then cease, no intimation being given of the anticipated result. It was a complete surprise when the involuntary contraction followed cessation of the voluntary effort. We have since learned that this phenomenon has been elicited for amusement by various groups of school children and it is stated by Pereira (1925) that this has been the case both in England and in Brazil. An army medical officer has reported that a considerable number of trials in the army unit to which he was attached revealed the presence of this phenomenon in about 70 per cent of the subjects tested. One of the most striking facts in the history of this phenomenon appears to be that it has regularly been elicited in the deltoid muscle, and indeed the impression is given that the deltoid is the only muscle which reacts in this way. As we shall presently show, the phenomenon is common to many muscles of the body including both flexors and extensors in the limbs (cf. Rothmann, 1915). That so many separate and apparently independent groups observing the phenomenon, have referred it to the deltoid, is remarkable.

Apparently, the first to make a scientific report of this phenomenon was Salmon (1914). Since his paper several others have reported observations on it. Recently Pereira (1925) has reported a series of experiments attempting to throw some light on the nature of the phenomenon. His experiments were performed in this laboratory, but their publication as a contribution from this department was not sanctioned, for the reason that the experiments were not adequately controlled and clearly did not warrant the conclusions which were drawn from them.

We have repeated the experiments, introducing the necessary controls, in order to determine whether Pereira's experimental results could be confirmed. It seemed especially important to settle this point, since it involves the question of action-currentless contraction in skeletal muscle. Many authors have maintained that there is a type of contraction in skeletal muscle, differing from ordinary voluntary contraction in the total absence of action currents; Pereira claims to have shown the phenomenon in question to be of this type. All previous claims to the demonstration of action-currentless contraction have been shown by Adrian (1925) to be invalid, with the possible exception of the peculiar phenomenon obtained under the influence of certain drugs by Lilljestrand and Magnus (1919). Any experiments, therefore, purporting to demonstrate the reality of action-currentless contraction should be carefully verified, if possible, or the nature of their fallacy sifted and revealed.

Pereira, applying electrodes to the skin over the deltoid muscle, claimed to have made the following observations. The post-contraction was regularly accompanied by small electrical excursions in the galvanometer, which he maintained were due, not to the action of the muscle, but to the mere motion of the arm; that is, not directly due to contractile effort but to the resulting change in shape of the muscle. In support of this contention he asserted that galvanometric excursions occur only when the arm is in motion, and that when it reaches its maximum elevation and ceases to rise the excursions cease, although the arm is maintained in an elevated position by active contraction of the muscle. He further stated that when the arm encounters an obstacle which prevents the deltoid muscle from raising it, the electric oscillations cease with the cessation of motion. When, on the other hand, the arm is passively raised by an external force, without contractile effort in the deltoid muscle, electric oscillations appear, closely resembling those which occur in the involuntary contraction of the muscle. This led him to associate the electric oscillations with motion *per se*, and not with contractile tension. Furthermore, he maintained that when the raising of the arm is voluntarily inhibited the electric oscillations cease, although he contended that the contractile tension continues, and the rise of the arm is prevented only by active contraction of the antagonistic muscles. He concluded that the origin of the phenomenon was purely muscular and without interference of the nervous system. In support of this view he adduced the further evidence that a brief isotonic contraction during the latent period of the "post-contraction" prevents its occurrence, and that the phenomenon could not be induced by isometric contraction with the arm in a horizontal position (i.e., with the muscle considerably shortened).

Assuming for the moment that Pereira's observations were correct, there are still logical weaknesses in the drawing of his conclusions, which

should be examined. The skin of the arm is at best a poor conductor of electricity, and action currents reaching the galvanometer from the muscle are correspondingly small. The tension of the galvanometer string used in his experiments was so great that small action currents, resulting from the response of only a few muscle fibers in unison, might well escape detection in the recorded electromyogram. The disappearance of galvanometric excursions, therefore, does not prove the total absence of the usual functional responses which are marked by the presence of action currents in the muscle. If in a particular experiment visible excursions ceased in the record at the moment when motion of the arm ceased, it would not necessarily prove the absence of the ordinary type of muscular contraction which is characterized by action currents. It might rather show that the weight of the arm could be supported without enough individual muscle fibers responding in unison to produce visible excursions in the electromyogram, under the conditions of recording; i.e., with tight string and high resistance in the circuit. Cessation of visible action currents when the arm encounters an obstacle, such as to arrest motion, would not prove that the contraction had not been initiated in the first place by motor-nerve impulses. Even if the action currents could be proved to have ceased altogether, this cessation might be explained by reflex inhibition of the contraction through proprioceptive impulses, unless evidence was furnished to show that active contraction persisted. Finally the presence of action currents when the arm is passively lifted by an external force is certainly no proof that these currents are independent of muscular effort and dependent on the deformation of the muscle through motion of the arm, as Pereira maintains, for there is no way of excluding the possibility that some voluntary muscles in the arm are actively contracting, even though the subject is endeavoring to keep them relaxed.

Thus, even supposing the experimental procedure were faultless, the results afford no real proof of the presence of action-currentless contraction in skeletal muscle, nor indeed of any essentially different type of contraction from that commonly resulting from voluntary effort. But there are reasons for questioning the validity of the experimental procedure as well. In Pereira's observations, only the action currents were recorded on the film; no simultaneous record was made of the mechanical effect of muscular contraction. The protocols contained a note of the contractions and relaxations of the deltoid muscle, but no adequate way was provided for correlating these motions with the changes in action current recorded on the film. It rested entirely with the judgment of the experimenter to select the portions of the record which corresponded with the rise and fall of the arm. To be certain that such experiments show a correlation of electrical oscillations with movement, as opposed to muscular effort, it would be necessary to register the motions of the arm and to establish

a method of synchronizing such a record with that of the action current simultaneously recorded. We have sought to repeat the procedures described by Pereira, adding the essential control; viz., making a simultaneous record of electrical and mechanical responses.

METHOD. We have used the Hindle string galvanometer which has been employed in this laboratory for several years. In this galvanometer we have used three different gilded quartz strings, one of 2.75μ diameter and 17,000 ohms resistance, another of 1.75μ diameter and 15,500 ohms resistance and a third of 2.25μ diameter and 11,000 ohms resistance. The recording camera has been described in a previous communication (Forbes and Thacher, 1920). Time is recorded on the film by means of a tuning fork vibrating 100 times a second. The galvanometer was connected with the subject by means of the same electrodes used by Pereira; strips of zinc wrapped with cotton moistened in zinc sulphate solution and strapped to the skin with surgeon's plaster. In most of our experiments the resistance of the circuit (including the electrodes and the tissues) was measured roughly by substitution, to make sure that absence of galvanometric excursions did not result from excessive dryness of the skin or poor electrode contact, with consequent excessive resistance. When the resistance was excessively high more solution was applied and the contacts were improved. Most of our observations were made with the deltoid muscle, but some were made with the biceps. The mechanical registration of the contractions of the muscle was simultaneously recorded on the film by the shadow of a wire so arranged, by means of strings and levers, that full motion of the arm, from extreme relaxation with the arm hanging at the side, to full elevation, produced an excursion just covering the width of the film.

Controlling our results by this method, we repeated all of the procedures employed by Pereira. The subject was seated on a stool, so placed that an arm, hanging vertically, was close to the leg of a heavy table. In the isometric contraction the back of the hand was pressed against the table leg. This was so placed that when, at the end of the isometric contraction, the arm was released it fell into a position from which it could rise without obstruction. With this arrangement we carried out the following procedures. The isometric contraction lasted in most experiments 20 to 25 seconds. Usually a photographic record of the action currents was made for the first few seconds of this period. Two seconds before the end of the isometric contraction the film was again started and a continuous photographic record was made including the entire course of the involuntary post-contraction. Then, after a rest in which the electrodes and other arrangements were not disturbed, the subject raised the arm by voluntary contraction of the deltoid at about the same speed as in the case of the involuntary contraction. In this way we obtained comparable electromyograms of the voluntary and involuntary contractions under identical

mechanical conditions and with the speed of contraction automatically recorded on the film, making it possible to verify the approximate equality of the force exerted in the two cases. This procedure was usually repeated two or three times with slightly varying speeds of contraction, in order to increase the chance of having a record which corresponded closely in this respect with the post-contraction. Next, in order to determine the effect of rendering the post-contraction isometric, a rigid obstruction was placed in the path of the arm to limit its rise, the electrodes and other arrangements being in no way disturbed. In some experiments the obstruction was so placed that the motion of the arm would be stopped when it had reached an angle of 20° or 30° from the vertical; in other cases it obstructed the arm at the outset. In these cases the records were taken as in the case of the unobstructed post-contraction, the film being started just before the cessation of the isometric contraction, and the motion of the arm as well as the electromyogram being recorded thereon.

The next procedure concerned passive motion. An assistant raised the arm at approximately the same speed as in the post-contraction by means of a string tied around the wrist, while the subject relaxed the muscles of the arm as completely as possible.

In the case of almost every subject tested, records were made while the subject voluntarily restrained the arm from rising. The question to be determined in these experiments was whether voluntary restraint consisted in a true inhibition of the post-contraction or in a contraction of the antagonistic muscles. The pectoralis major is the chief antagonist of the deltoid. These muscles have one advantage over the biceps and triceps for this experiment, because the two latter muscles lie so close together in the arm that it is impossible, with externally applied electrodes, to differentiate with certainty between their action currents; i.e., action currents in the triceps would be recorded with leads applied over the biceps and vice versa. The pectoralis major, on the other hand, is so remote from the deltoid muscle that the action current of one could scarcely be recorded through leads applied to the other. This eliminates, or at least minimizes, an important source of confusion. In the case of four subjects, leads were applied over the upper edge of the pectoralis major and the experiment of voluntary restraint of the post-contraction of the deltoids was repeated. For purposes of comparison, the electromyogram of a maximal voluntary contraction of the pectoralis major was recorded.

In all, seven subjects were tested. With two of these we repeated Pereira's two other procedures, one was to intercalate a short, voluntary isotonic[1] contraction immediately after the cessation of isometric contrac-

[1] Throughout this paper we use the term *isotonic contraction*, as Pereira did, to signify raising the arm without obstruction or load, other than its own weight. We recognize that this is not strictly isotonic, since the load increases as the arm is raised from the vertical to the horizontal position.

tion and during the normal latency of the post-contraction. The other was to make the voluntary isometric contraction with the arm already raised to a horizontal position, so that the deltoid muscle was considerably shortened, instead of in the usual vertical position with the deltoid muscle at its maximum length.

Results. Of seven subjects tested, one failed to show the involuntary post-contraction at all. Another showed it only feebly. In the remaining five it occurred with perfect regularity. One of these was unacquainted with the phenomenon, and was given no intimation of what to expect. With this subject a record was obtained of the first occurrence of this phenomenon in his experience, which therefore furnishes an electromyogram of a wholly unexpected contraction (fig. 4B).

In all of our procedures, except those dealing with the question of voluntary restraint of the post-contraction, our results were perfectly uniform. They consistently showed, as far as the refinement of the method made it possible, a perfect quantitative correlation of action currents with contractile effort in the involuntary post-contraction, as well as in ordinary voluntary contraction, whether the arm was in motion or was restrained from rising by an obstruction or by its own weight. There was no indication whatever that action currents are correlated with motion as such, rather than with active contraction of the muscle. On the contrary, our results regularly show the action currents to be related to active contraction irrespective of motion.

Let us consider the evidence in detail. In figure 1 is shown a series of records illustrating all the significant stages of the unobstructed post-contraction in a typical experiment. The first part, A, shows the voluntary isometric contraction when the hand was being pressed against the leg of the table with nearly the full strength of the muscle. The second part, B, shows the beginning of the post-contraction, and it will be seen here that the action currents begin with the motion of the arm. The third part, C, begins just before the arm reaches the horizontal position and shows the electromyogram of the involuntary contraction holding it in that position. It will be seen that although the arm ceases to move, action currents still persist during the contractile effort of the muscle required to hold the weight of the arm in a horizontal position. Figure 1D shows a voluntary contraction of approximately the same strength as that which raised the arm in the involuntary post-contraction. It will be seen that there is no significant difference between the electromyograms in the two cases. Results similar to this were regularly obtained in every one of the subjects tested.

Pereira laid great stress on the observation that no action currents were visible in his records after the arm ceased to move in the post-contraction; i.e., while the involuntarily contracting muscle was holding the arm

motionless against gravity. This statement is refuted by the record in figure 1C. Furthermore, in figure 2 we have reproduced five records of the corresponding (motionless) phase of involuntary contraction from five

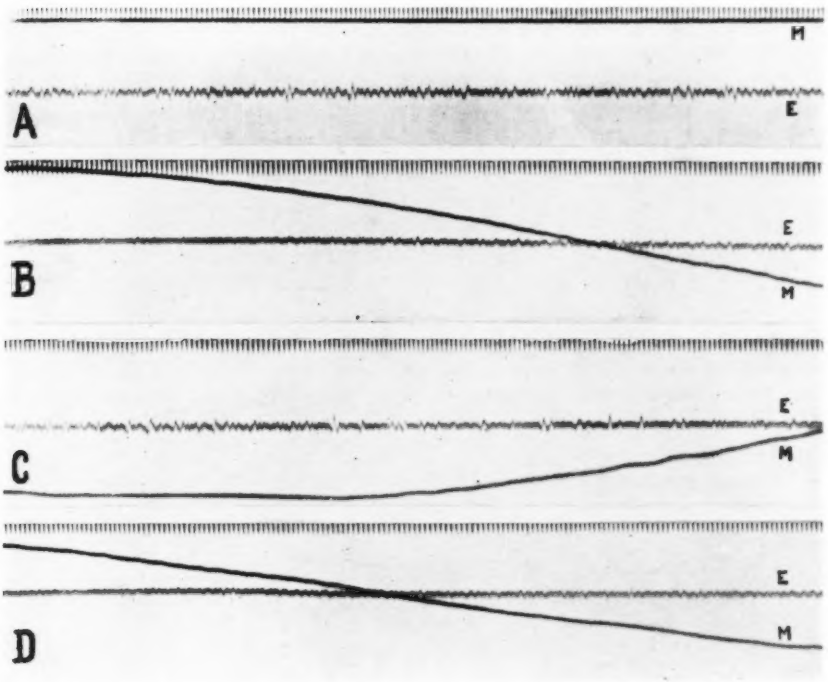


Fig. 1. Electromyograms, *E*, and mechanograms, *M*, showing typical case of well-developed post-contraction, and voluntary contraction for comparison. Deltoid muscle; February 16, 1926; subject, P. C. B.; 2.25μ string; tension, 160 meters per ampere (see Forbes and Ray, 1923).

A, during strong isometric contraction; B, beginning of post-contraction; C, sustained maximum and decline of post-contraction; D, control record of unrestricted voluntary contraction ("isotonic") for comparison with B.

In all figures fall of mechanogram line signifies contraction of muscle. Time shown above by tuning fork shadow, 1 d.v. = 0.01 second. Magnification of galvanometer string 490.

separate experiments taken on four different subjects. These are all typical electromyograms, practically indistinguishable from those of voluntary contractions of the same strength. As far as the action currents are concerned, it appears to make no difference whether the arm is

in motion or not. The electrical record during this stationary period does not differ visibly from that occurring in the rising phase, except that the action-current frequency appears to decrease just before the arm begins to fall; throughout its course it resembles closely the record of a voluntary contraction of the same strength under the same mechanical conditions.

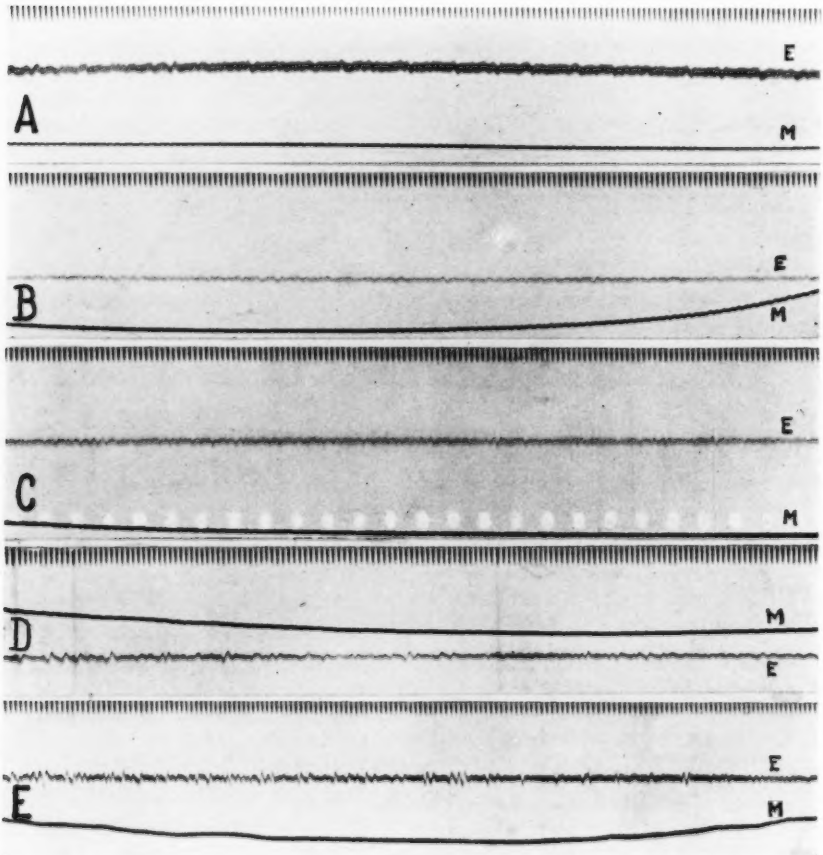


Fig. 2. Sustained maximum of post-contraction in five different experiments, showing persistence of action currents after cessation of motion. Deltoid muscle. All experimental conditions as in figure 1.

A, subject, P. C. B.; November 24, 1925; 275μ string; tension 138 m. per amp. B, subject, A. F.; December 8, 1925; 1.75μ string; tension 127 m. per amp. C, subject, A. M. P.; December 8, 1925; 1.75μ string; tension 127 m. per amp. D, subject, A. H.; December 15, 1925; 1.75μ string; tension 158 m. per amp. E, subject, P. C. B.; February 16, 1926; 2.25μ string; tension 160 m. per amp.

The effect of rendering the involuntary post-contraction isometric by placing a rigid obstruction in the way of the moving arm is shown in figure 3. The uppermost record, A, shows the electromyogram during the ordinary unrestricted rise of the arm. It is taken from the same experiment that furnished the records in figure 1, and is a repetition of the observation shown in figure 1B. In figure 3B, the rise of the arm was blocked by a rigid obstruction at an angle of about 20°. The mechanical record clearly shows the cessation of motion and reveals the fact that the arm, instead of

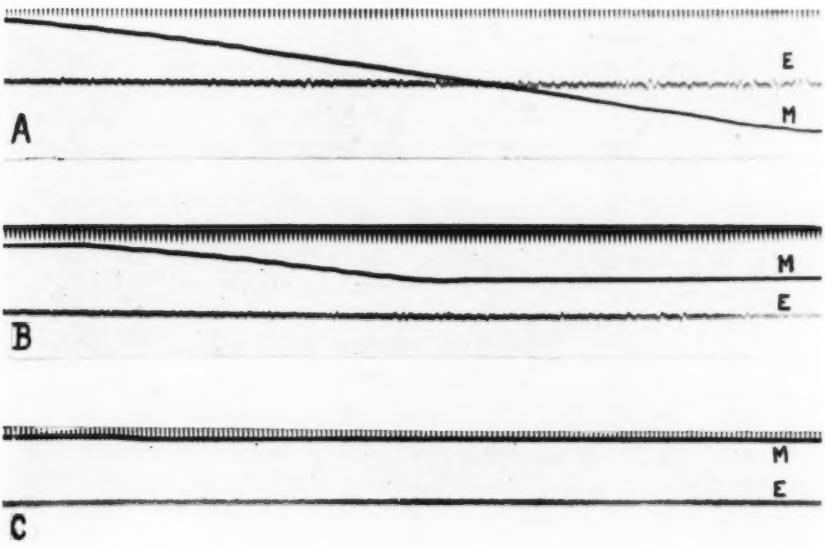


Fig. 3. Electromyograms and mechanograms showing effect of obstructing the post-contraction at different stages, and persistence of action currents as long as contraction persists. Deltoid muscle; subject, P. C. B.; February 16, 1926. A, unobstructed; B, obstructed when arm has risen to angle of about 20°; C, obstructed at outset. 2.25μ string; tension 169 m. per amp.

falling back, remained held in contact with the obstruction by active contraction of the muscle. It will also be seen that with cessation of movement there was no cessation of action currents; the electric responses of the muscle continued essentially unchanged after the arm had been prevented from rising, and indeed show a slight increase in amplitude during the first half second after motion ceased. Similar results were obtained in a large number of such tests on every one of our subjects who manifested the post-contraction. Our results, therefore, regularly contradict the

statement of Pereira that action currents cease with motion, even though the muscle continues to exert a contractile effort. Figure 3C is a record of the post-contraction obstructed at the outset. In this case the subject was so placed that when the deltoid started to contract, the hand at once encountered the same table leg against which it had been pressed during the isometric contraction. The subject noted that the usual tendency of the arm to rise ceased abruptly when the obstruction was thus encountered. Corresponding with this absence of a well-developed post-contraction is an absence of visible action currents. The mechanical record shows the beginning of the post-contraction as a very slight excursion of the recording lever, and thus reveals how closely limited was the motion of the arm. The difference revealed in the comparison between records B and C in figure 3 was found to be fairly constant; that is, when the post-contraction was allowed to raise the arm to any considerable distance, e.g., to an angle of 15° or 20°, the involuntary contractile effort persisted and continued to hold the arm against the obstruction for several seconds; whereas, when the obstruction prevented the arm from beginning to rise, relaxation followed promptly. In every case the action currents were closely correlated with the contractile effort, persisting as long as the muscle contracted, and ceasing when it ceased to contract. Under these conditions, as under the conditions shown in figure 1, there was no evidence whatever of the alleged exclusive correlation of action currents with motion as such, but, on the contrary, there was a regular correlation of action currents with active contraction, whether isometric or isotonic.

Pereira supported his contention that the electric disturbances accompanying post-contraction were associated with motion of the arm as such, by showing similar disturbances in a case in which the arm was passively raised by an assistant, while the deltoid muscle remained supposedly relaxed. In our experiments, as in Pereira's, action currents sometimes appeared when the arm was raised by an assistant, but they were always small compared with those appearing when the arm was raised at the same speed by active contraction of the deltoid muscle. In at least one instance, passive raising of the arm was unaccompanied by visible galvanometric excursions, although immediately before and afterwards active contraction was marked by clearly visible action currents in the record, thus showing that their absence during the passive lift could not be explained by any defect in the recording circuit. An illustration of this is shown in figure 4. The upper record was made during the passive lifting of the arm by an assistant. The middle record shows the electromyogram during a rather weak post-contraction. The bottom record shows, for comparison, a normal isotonic contraction; that is, the voluntary raising of the arm at about the same speed as in the case of the passive lift. This set of records, showing the complete absence of visible action currents during passive

motion and the close similarity of the electrical records in the case of the involuntary post-contraction and in that of voluntary contraction, makes it clear that action currents are a feature of activity in the muscle and are not due to motion of the arm as such. The fact that in some records action currents appear when the arm is passively lifted has no special significance in this connection, for it is most difficult for the subject by any voluntary effort to insure complete relaxation of the deltoid muscle and of others in the vicinity. With the electrodes applied to the skin, contraction of any

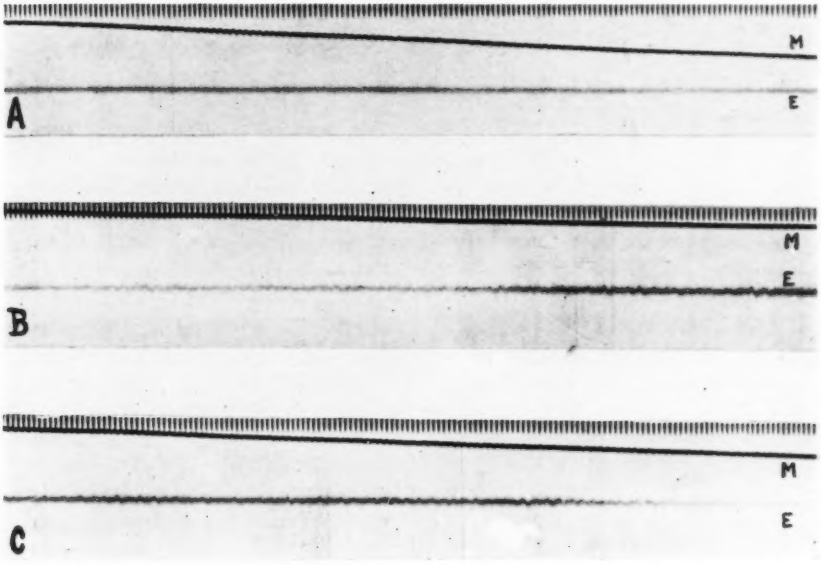


Fig. 4. Records of passive lift compared with weak post-contraction and voluntary contraction. Deltoid muscle; subject, D. T.; December 10, 1925.

A, passive lift, muscles relaxed; B, weak post-contraction, showing action currents; C, weak voluntary contraction, no load. 1.75μ string, tension 158 m. per amp.

of the muscles of the upper arm would cause action currents to appear in the record.

It is clear then that action currents are the regular accompaniment of the active contractile process in the case of the involuntary post-contraction, as well as in that of voluntary contraction, and are not to be regarded as a secondary effect due to the mechanical shortening of the muscle. In all of our experiments the records were perfectly uniform in pointing to this conclusion.

When we repeated Pereira's two other procedures, mentioned above,

with which he endeavored to reinforce his conclusions, we obtained results diametrically opposed to those which he reported. Intercalating a short voluntary unrestricted contraction in the normal latency of the post-contraction did not prevent the appearance of the latter, nor did it materially decrease its magnitude. The occurrence of the post-contraction after this procedure was shown repeatedly. When the voluntary isometric contraction was made with the arm already raised to the level of the shoulder, instead of hanging at the side, the involuntary post-contraction occurred as usual and did not appear to be noticeably less than in the other case.

In attacking the question whether voluntary restraint of the post-contraction was effected by true central inhibition or by contraction of the antagonistic muscle, our results were not so uniform as in the previous procedures. In most of the subjects there seemed to be true inhibition of the deltoid muscle. This is inferred by the partial or complete cessation of action currents when the post-contraction was restrained. We soon found that it was much easier to exercise true inhibition when this was done at the outset before the arm had begun to rise. If voluntary restraint was not begun until the arm had risen to a considerable angle, more effort was required to stop it, and the action currents did not cease completely. Typical evidence of this is shown in figure 5. The first three records in this figure were taken with the leads on the deltoid muscle; the last three with the leads on the pectoral. The first record shows the unrestrained post-contraction; the second shows the effect of inhibiting after the arm had risen to a considerable angle. It will be seen here that small action currents persist, although they are considerably smaller than in the unrestrained post-contraction; there must have been a partial inhibition in this case. The third record shows the effects of inhibiting the post-contraction at the outset. Here action currents are absent, and the inhibition appears to have been complete. The fourth record, with the leads on the pectoral, shows voluntary restraint after the post-contraction had started. The next record shows voluntary inhibition at the outset. In neither case are action currents in the pectoral muscle visible in the record. The bottom record, also with the leads on the pectoral, shows the electromyogram of a powerful voluntary contraction of this muscle. This shows clearly the type of action currents which could be obtained from a maximal contraction, and makes it evident that little or no pectoral activity was involved in restraining the post-contraction of the deltoid (fig. 5D). It is interesting to note that the action-current rhythm in the case of the pectoral muscle was slower and more regular than in the case of the deltoid. This was so in all subjects tested.

In the case of one subject, the first attempt to restrain the post-contraction by voluntary effort involved a sense of contractile effort in the an-

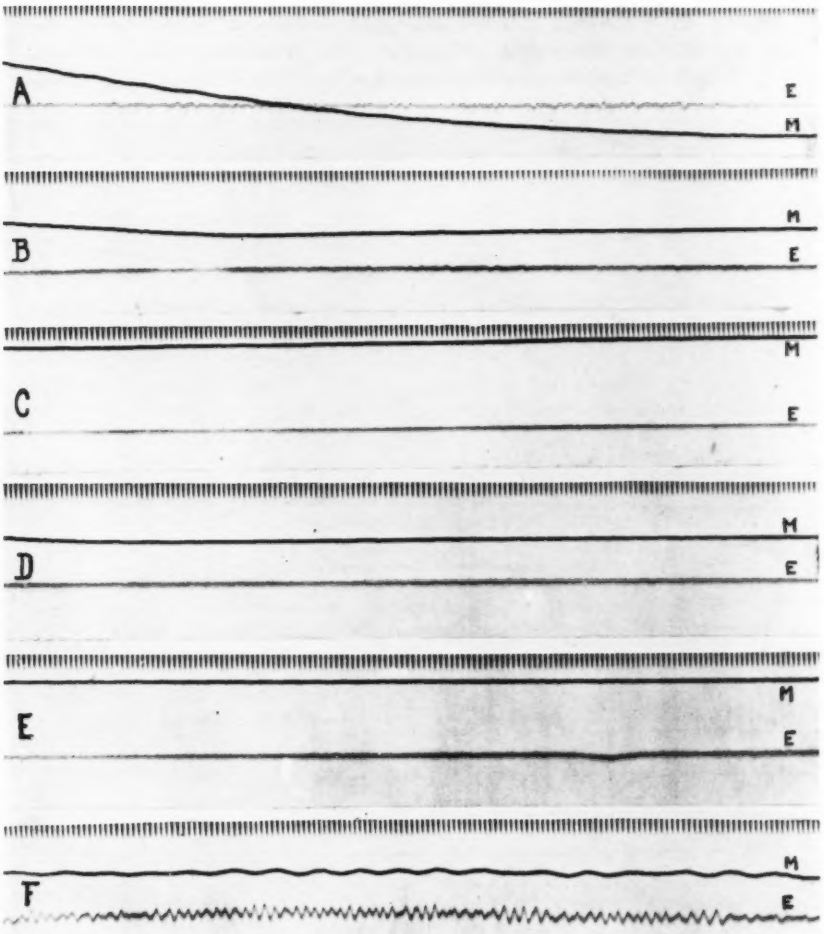


Fig. 5. Records showing voluntary restraint of post-contraction. Subject, P. C. B.; December 15, 1925.

A, post-contraction, unrestricted; B, post-contraction restrained at angle of about 20° by voluntary inhibition; C, post-contraction restrained at outset, voluntary inhibition. In A, B and C, leads applied to deltoid muscle. In D, E and F, leads on pectoral muscle. D, post-contraction of deltoid restrained at angle of about 20° by voluntary inhibition, as in B; E, voluntary inhibition at outset, as in C; F, electromyogram of strong contraction of pectoral for control. 1.75μ string, tension 158 m. per amp.

tagonistic muscles. That the post-contraction was restrained by active contraction of the pectoral, and not by true inhibition, seems to be indicated by the electromyograms shown in figure 6, taken from this subject. The first record shows the electromyogram of the deltoid in the unre-

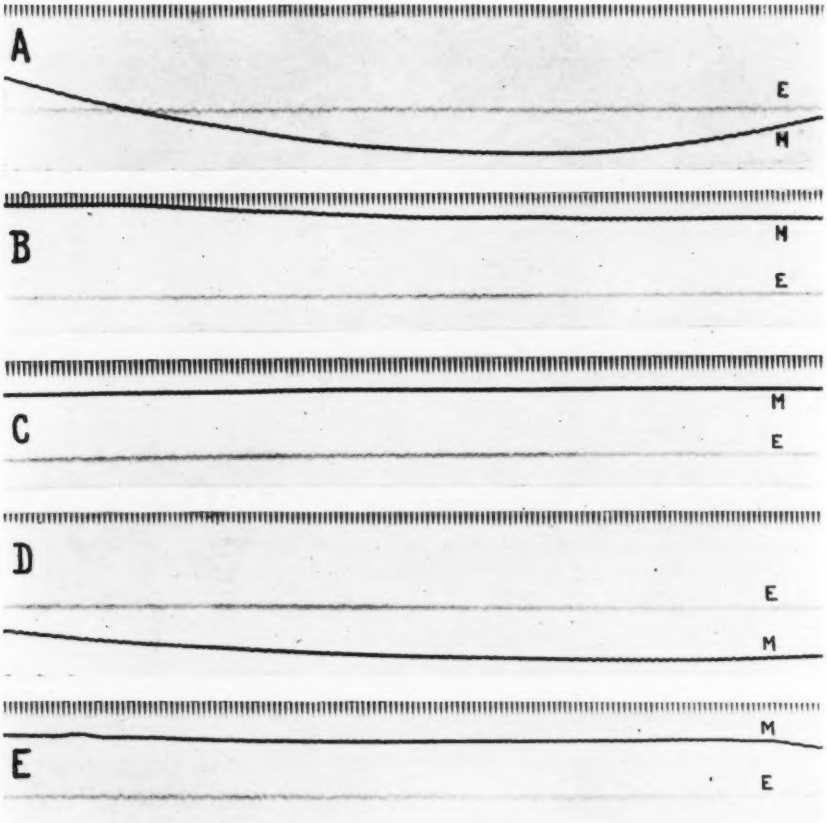


Fig. 6. Records showing voluntary restraint of deltoid post-contraction, apparently involving contraction of pectoral muscle. Subject, A. F.; December 8, 1925. A and B, leads on deltoid; A, unrestricted post-contraction; B, voluntary restraint early in rise; C, D and E, leads on pectoral; C, voluntary restraint early in rise; D, unrestricted post-contraction; E, strong contraction of pectoral. 1.75μ string; tension 127 m. per amp.

strained post-contraction. The second record shows the attempt to inhibit, also with leads on the deltoid. The third record, with leads on the

pectoral, shows the presence of action currents when the post-contraction was restrained and seems to indicate that a contraction of the pectoral was required. The fourth record shows an almost complete absence of action currents in the electromyogram taken from the pectoral during the unrestrained post-contraction of the deltoid. There is no reason to suppose that the pectoral muscle was contracting, and the small excursions are probably due to the action currents in the deltoid; thus they serve to show the small extent to which deltoid action currents may be derived from leads applied over the pectoral. The bottom record shows the electromyogram of a maximal contraction in the pectoral muscle in this subject, the conditions being the same as in the bottom record in figure 5. The unusually small excursions in figure 6E probably signify an unusually high resistance in the circuit, which in this instance was not measured.

An interesting observation was made in the case of this subject. Although his first attempt to restrain the post-contraction appeared to result in active contraction of the antagonistic muscle, after a few attempts he seemed to learn to exercise inhibition, for thereafter records were made in which the action currents disappeared, as in the case of other subjects. With him, as with the other subjects, it was noted that inhibition was much easier if begun before the post-contraction had started to move the arm. In this respect there was a close resemblance between voluntary inhibition and mechanical restraint, for it will be recalled that when the arm was obstructed at the outset of the post-contraction muscular activity ceased; whereas, when the arm was allowed to rise to a considerable angle, both contraction and action currents continued after the obstruction was encountered.

From the foregoing it will be seen that the majority of our experiments on voluntary restraint of the post-contraction failed to confirm Pereira's findings. He contended that restraint was only possible by active contraction of the antagonist. We find, on the other hand, that in a majority of cases true inhibition can be achieved.

Salmon (1925), in his interesting paper on this subject, contends that there is little or no correlation between the effort expended in the voluntary isometric contraction and the resulting intensity or duration of the post-contraction. In support of this contention he cites certain individuals who show a marked post-contraction after comparatively little previous voluntary effort, and other individuals who show little or no post-contraction after great voluntary effort. Undoubtedly, Salmon is correct in this observation, but in our experience there is a fairly striking correlation *in a given individual* between the amount of exertion in the voluntary isometric contraction and both the intensity and the duration of the resulting post-contraction. Usually we employed a voluntary isometric contraction not quite maximal in strength and lasting only 15 to 25 seconds.

The resulting post-contraction usually raised the arm approximately to the horizontal position (sometimes not so high) and held it there from $\frac{1}{2}$ second to 10 seconds after the arm had ceased to rise.

In some experiments on two subjects we tried the effect of a great in-

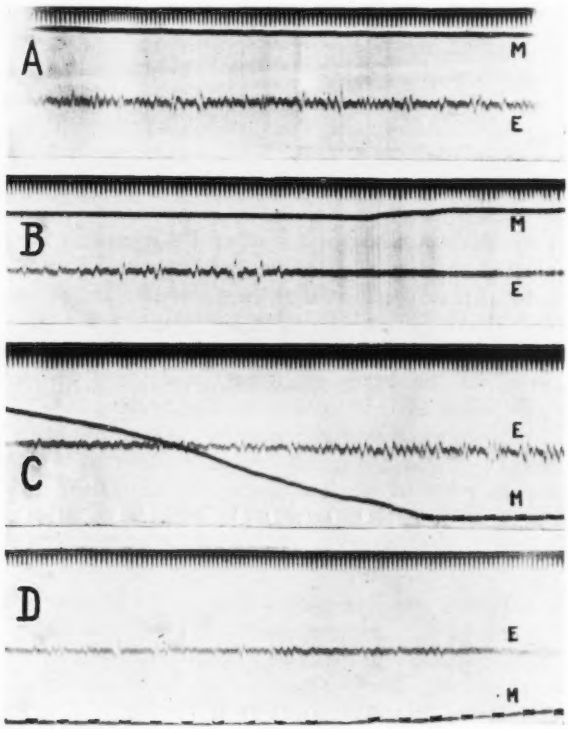


Fig. 7. Records showing increased strength and duration of post-contraction following prolonged isometric contraction with extreme exertion. Subject, P. C. B.; deltoid muscle; February 16, 1926.

A, electromyogram early in maximal isometric contraction; B, conclusion of isometric contraction lasting 1 minute; C, first stage of post-contraction, showing rapid rise of arm and large action currents; D, first relaxation of post-contraction, beginning 4 seconds after end of C. Action currents well sustained throughout motionless phase of post-contraction. 2.25μ string; tension 160 m. per amp.

crease in the strength and duration of the voluntary isometric contraction. Each of these subjects contracted the deltoid muscle as hard as he was able for a full minute. The resulting post-contraction was most striking. The arm rose to a horizontal position and remained there for much longer

than usual. In each case the arm started to fall and rose again, repeating this performance two or three times before the post-contraction ceased altogether. The total duration of the post-contraction was in one case 25 seconds. Records from one of these experiments are reproduced in figure 7. The top record shows the beginning of the extreme isometric contraction. The second record shows its termination. The characteristic effect of fatigue reported by Piper (1912) and examined in more detail by Cobb and Forbes (1923) is revealed in the comparison of these two. The third record shows the strong post-contraction just as it was reaching its maximum. The bottom record shows the later stages of the post-contraction just before relaxation occurred and when the action current rhythm was becoming appreciably slowed. In figure 7C it will be seen that the action currents in the post-contraction were in this case considerably larger than in those following the shorter and less powerful isometric contractions (figs. 1 to 3).

In the introduction we noted that this phenomenon has usually been observed only in the deltoid muscle. We have found it to occur in many muscles in various parts of the body. Among these are the biceps, the triceps, the wrist flexors, the abductors and flexors of the hip, the flexors and extensors of the knee, and the muscles of the neck. The tendency seems to be universal in skeletal muscles, requiring only suitable mechanical conditions to render the effect visible.

Figure 8 shows a series of records obtained with leads applied to the upper arm over the biceps muscle. This series embraces nearly all of the procedures shown in the previous figures in the case of the deltoid. The top record, A, shows the electromyogram of the isometric contraction. Record B shows the post-contraction and its attendant action currents persisting after motion has ceased, as long as the forearm is held up against gravity (as in the case of the deltoid). Record C shows a voluntary isometric contraction for comparison. Judging by the speed of flexion the contraction must have been slightly stronger than the post-contraction shown in record B, yet the action currents are little or no larger. If the post-contraction depended chiefly on an action-currentless mechanism, as contrasted with voluntary contraction, its electromyogram should show much smaller excursions, if any, than those of voluntary contraction. But this is clearly not the case.

Record D shows the effect of a passive lift. The electrical oscillations here are very much smaller than in the case of either of the above active contractions, and as in the case of the deltoid, these small excursions are of no great significance, since it was impossible to insure complete relaxation of all muscles in the upper arm. Record E shows the result of voluntary restraint of the post-contraction after flexion had progressed through a considerable angle; record F shows voluntary restraint at the outset.

In the former, E, it will be seen that small galvanometric excursions appear with the beginning of post-contraction, comparable in size with those in the corresponding stage of the unrestrained post-contraction, B. With restraint they soon die away and become almost imperceptible. When restraint was begun at the outset, F, action currents did not appear. The bottom record, G, was made by a strong isometric contraction of the triceps with the leads still over the biceps. This shows to what extent action currents from the triceps can appear in the record with this arrangement.

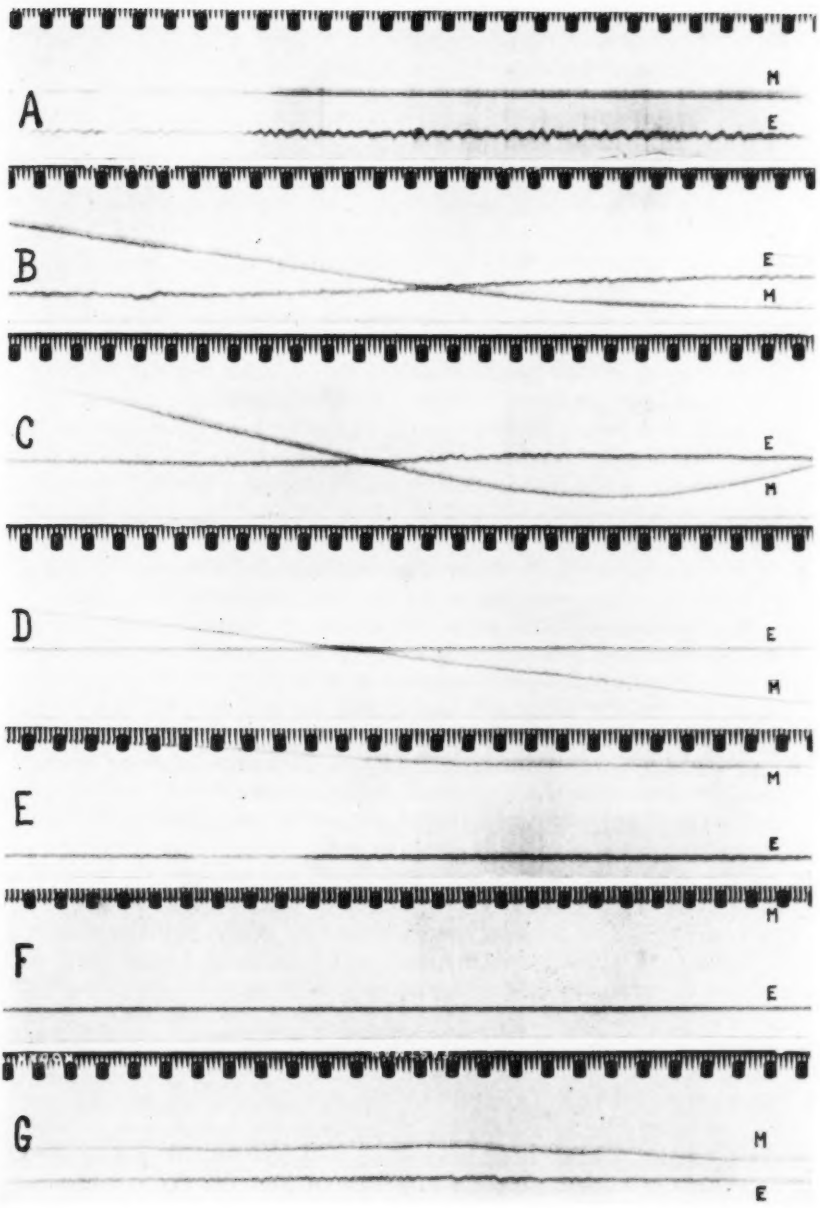
The results in these records of voluntary restraint agree with most of those obtained from the deltoid. True inhibition is more easily achieved when the post-contraction is not allowed to begin. When the post-contraction is permitted to progress till the muscle has shortened appreciably, the contraction persists a short time, and the action currents continue long enough after motion is stopped to suggest that contraction of the antagonist plays a part in arresting the movement; but their small size and rapid subsidence lead to the view that a true inhibition supervenes.

In general the results obtained with the biceps agree with those obtained with the deltoid. Every procedure employed with both deltoid and biceps showed that action currents are the regular concomitant of the post-contraction, whether the limb is allowed to move or not, furthermore, the apparent amplitude and rhythm of the galvanometric excursions are always approximately the same as in the case of a voluntary contraction of the same strength. The uniformity of these results seems to dispose effectually of the theory that a special action-currentless type of contraction is involved in this phenomenon. The involuntary post-contraction is, by every objective test we have employed, indistinguishable from voluntary contraction.

Discussion. Two general theories have been advanced concerning the nature of involuntary post-contraction; one that it originates in the muscle and does not depend on motor-nerve impulses; the other, that it is caused by some sort of central excitation and is evoked by nerve impulses, as is a voluntary contraction. Pereira advocates the former view for reasons which would not have been altogether valid, even if his observations had been correct, and which may now be disregarded, in view of the more rigidly controlled observations here reported. Salmon (1925) makes a good case for the theory of central innervation. He proposes an interesting

Fig. 8. Records of post-contraction in biceps muscle. Subject, A. F.; December 15, 1925.

A, voluntary isometric contraction; B, rising phase of post-contraction; C, voluntary isotonic contraction; D, passive lift with muscles relaxed; E, voluntary restraint after post-contraction has begun; F, voluntary restraint at outset, inhibition apparently complete; G, maximal, voluntary isometric contraction of triceps, leads still on biceps. 1.75μ string; tension 158 m. per amp.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hypothesis to the effect that after the prolonged voluntary effort a persistent representation of the act remains in the cerebral motor cortex and causes continued discharge of nerve impulses from the center involved. He likens this to the persistence of sensory representation after an intense sensation, such as a sight or a sound. It is doubtful how far this analogy is justified, especially in the case of visual sensations, in which afterimages are certainly in large measure of retinal origin.

So far as we are aware no theory yet proposed has taken into consideration the probable rôle played by proprioceptive impulses in this phenomenon. Numerous researches from Sherrington's laboratory (Sherrington, 1909, 1915; Liddell and Sherrington, 1924; Fulton and Liddell, 1925) have recently brought to light the large part played by these afferent nerve impulses of intramuscular origin in reflex coördination. The impulses themselves have been recorded by Forbes, Campbell and Williams (1924) and more recently by Adrian (1926); and it has been shown that the stimulus which evokes some, at least, of these proprioceptive impulses is tension applied to the muscle. There is reason to believe that there are several types of sensory receptors in skeletal muscles, and that they are adapted to different kinds of stimuli. It is, therefore, natural to suppose that some aspect of the prolonged isometric contraction might well constitute a persistent source of excitation to at least one of the various types of receptors. This seems to us the simplest explanation of this rather baffling phenomenon. We would not attempt to identify the particular histological type of receptor involved, nor to explain just how it is stimulated by the after-effects of prolonged contraction. But that some physical or chemical effect may arise and provide the necessary stimulus is not improbable.

In support of this hypothesis we may emphasize the following points. That the post-contraction is of central origin is rendered highly probable by its close similarity to voluntary contraction, and by the absence of any valid reason for supposing it to be of purely muscular origin. That the stimulus which evokes it comes from the muscle itself, and not from the cerebral cortex, is strongly suggested by the two related observations we have made on its restraint, both voluntary and mechanical. We found that when the post-contraction is prevented from moving the limb, either by an obstruction or by voluntary inhibition, the contractile effort ceases promptly. But when the limb is allowed to move a considerable distance before being stopped, the post-contraction does not cease so readily. In the case of mechanical obstruction, the muscle continues to contract for some time after motion is blocked. In the case of voluntary restraint, activity persists in the muscle for a short time after its motion ceases, and in some cases, at least, it appears that the antagonistic muscle must take part in checking the motion. These results suggest that the post-contraction

tends to build itself up; if checked at the start, it dies easily; if contraction is allowed to become established, it tends to maintain itself. It is difficult to picture any mechanism which could cause such behavior, other than reflex excitation in which the afferent impulses arise in the muscle. Since tension is known to be a stimulus for some intramuscular receptors, it is easy to see how when the muscle begins to establish tension by its contraction, the reflex should tend to augment itself. This hypothesis involves few doubtful assumptions, and as far as we can see, is in accord with virtually all the facts now established concerning this phenomenon. Salmon's observation (1916) that the effect is diminished in tabes dorsalis tends to support this view. His further observation that although diminished, it persists in severe cases of tabes, in which tendon reflexes are absent, tends to throw doubt on this hypothesis, but does not disprove it, for in these cases there is no certainty that all proprioceptive impulses were absent. The failure of the post-contraction to appear after faradization of the muscle (Siciliano, 1914; Kohnstamm, 1915) presents an apparent difficulty, for one would expect artificial tetanus to affect the intramuscular receptors in the same way as voluntary contraction. But faradization of the muscle probably excites the afferent fibers in its motor nerve, and might thus induce a synaptic fatigue in the reflex arc (cf. Forbes, 1912), which would account for the difference in after-effect.

It should be understood that when, in describing our results, we have mentioned variations in the size of action currents or in the completeness of inhibition, nothing is implied which is contradictory to the all-or-none law of muscle response. The amplitude of an observed excursion is taken to depend on the number of fibers responding approximately in unison at the moment. Furthermore, the observed rhythm is not necessarily assumed to represent the actual rhythm of response in any individual muscle fiber; the record is recognized as a composite picture derived from a complex aggregate of fibers. For further consideration of the interpretation of the electromyogram we may refer to the papers of Adrian (1925), Forbes and Cattell (1924), Forbes and Olmsted (1925), and other papers referred to therein.

SUMMARY

1. Salmon in 1914 described a tendency of skeletal muscles in man to contract involuntarily upon cessation of a prolonged, intense, isometric voluntary contraction. Many people have observed this "post-contraction," and apparently it occurs in about 70 per cent of those tested. That it is not due wholly to mental suggestion is shown by the fact that we have observed it in three subjects who had no previous intimation that such a contraction was to be expected.

2. A number of investigators have studied this phenomenon; some have concluded that it involved only the muscle and was not evoked by motor-nerve impulses; others assign the cause to persistent discharge from the

nerve centers. Pereira, in support of the muscular theory, reports observations which led him to the conclusion that the post-contraction is due to an action-currentless activity, wholly distinct from the ordinary contraction evoked by nerve impulses. To show that it is not controlled by the nervous system, he contends that its voluntary restraint is only achieved by contraction of an antagonistic muscle.

3. We have repeated Pereira's experiments on seven subjects, introducing a mechanical device for recording motion of the limb on the film with the electromyogram. We find that action currents regularly accompany the post-contraction, whether the limb is in motion or not. When the arm ceases to rise, either because of an obstruction or because of its own weight, action currents persist as long as contractile effort continues. The action currents show no significant difference in amplitude or rhythm from those of voluntary contraction of the same strength. The electric oscillations in passive motion are very much smaller and are sometimes absent; when present they are readily explained as due to incomplete relaxation of the muscles in the arm. Thus there is no valid ground for associating electric excursions with motion as such; they are due to action currents and are regularly correlated with active contraction. We find that intercalating a brief isotonic contraction does not prevent the occurrence of the post-contraction; we also find that it may be induced by an isometric contraction with the arm in the horizontal position, as well as in the usual vertical position.

4. Voluntary restraint is easily achieved by inhibition at the outset of the post-contraction; when contraction has been allowed to begin, inhibition becomes more difficult and the antagonistic muscles are probably called into play, in some cases, to check the contraction. Similarly, a rigid obstruction, preventing the beginning of the post-contraction, causes prompt relaxation, whereas obstruction after the limb has moved through a considerable angle, is met by continued isometric contraction for a time.

5. There is no reason to conclude that a special action-currentless mechanism is involved in this phenomenon, nor any other neuromuscular apparatus than that employed in voluntary contraction. The objective similarity of the post-contraction to voluntary contraction is so close that there is every reason to suppose that the nature of the neural discharge from the spinal nerve centers is essentially the same in both. It seems to us that the simplest explanation of the post-contraction is that the motor-neurone discharge is reflexly evoked by proprioceptive impulses, which are in some way set up by stimulation of intramuscular receptors in consequence of some as yet undetermined effect of the prolonged isometric contraction.

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Page 103: The bibliographical reference to the article by J. R. Pereira should read "1925. Journ. de Physiol. et de Pathol. Gen., xxiii, 30" instead of "1925. Journ. Physiol., xxiii, 30."

CONTRIBUTIONS TO THE PHYSIOLOGY OF GASTRIC SECRETION

XI. The Effect of Ethylene Anesthesia on Gastric Secretion and Motility

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This work was undertaken for several reasons. In the first place, an anesthetic that would not alter gastric secretion and motility would be of great assistance to those investigating these subjects. Because of the usual lack of symptoms following, and the relative low toxicity of ethylene, we hoped that ethylene might prove to have no effect on gastric secretion. In the second place, since a good ethylene anesthesia resembles normal sleep in many ways, we desired to ascertain if gastric secretion was affected similarly by these two procedures. In the third place, if ethylene does not markedly disturb gastric secretion and motility, one has an additional fact favoring the employment of ethylene as a general anesthetic.

Methods. Men were used in our experiments because it is known that they are more susceptible to ethylene anesthesia than dogs (Luckhardt, 1925). Normal men were studied by the usual technique employed for gastric analysis. The acidity of the gastric juice is expressed in clinical units.

The emptying of the stomach was studied fluoroscopically quite accurately by having the subject and screen a fixed distance from the tube. Tracings of the stomach were then made on the screen with a glass pencil and copied on paper. By this means it was possible for us not only to follow the time of emptying, but also the rate at different half-hour periods.

The period of ethylene administration was always five minutes as we were not directly interested in the effects of prolonged anesthesia.

Results. *Gastric secretion.* The continuous gastric secretion before and after five minutes of ethylene administration was followed in eight subjects. In four subjects there occurred a temporary (10 to 20 minutes) disappearance of free acid from the continuous secretion followed by a gradual rise to subnormal values. In three subjects free acid was abol-

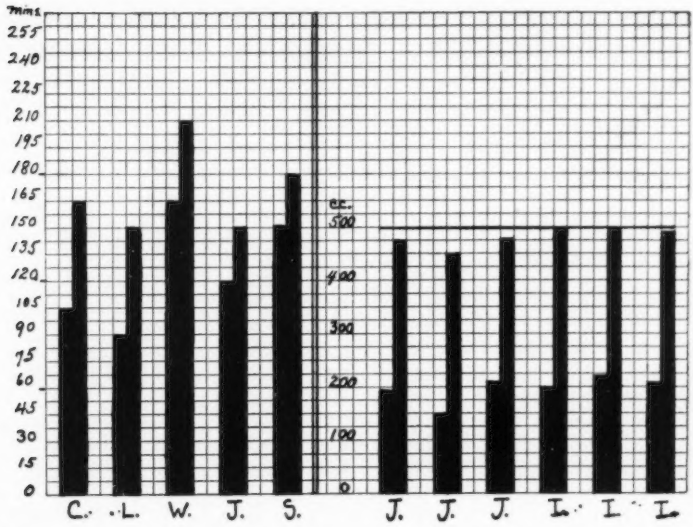

Fig. 1. The ordinates on the left represent emptying time in minutes for subjects C, L, W, J and S. The ordinates in the center represent the volume of water aspirated from the stomach in three experiments with subject J, and three experiments with subject I twenty minutes after 500 cc. water were drunk. In every case the lower column is the control, the higher column immediately following is the data obtained when ethylene was administered. See tables 2 and 3.

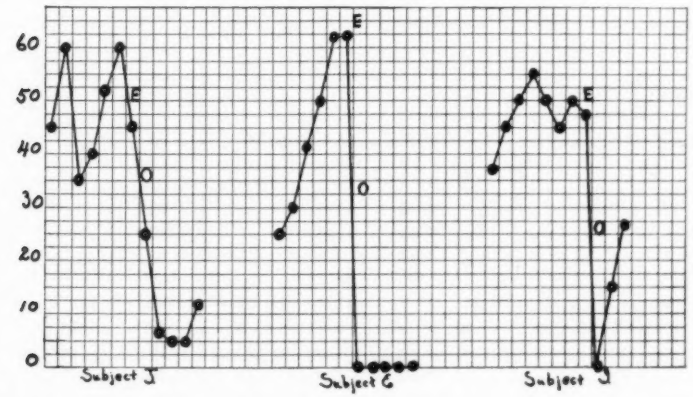

Fig. 2. In these three experiments the Rehfuss tube was taken into the fasting stomach. The dots indicate the free acidity of specimens taken at ten-minute intervals. The preliminary rise is due to the mechanical stimulation of the tube. *E*, administration of ethylene; *O*, removal of ethylene mask. Note that the acidity remains down for a time after removal of ethylene. The ordinate figures at the left indicate acidity in clinical units.

ished from the secretion for from 40 to 60 minutes, which was as long as we continued the experiments. In one subject, subject J, free acid was

TABLE 1

Effect of ethylene anesthesia on gastric secretion. Normal men

PROCEDURE	TIME O'CLOCK	AMOUNT	GASTRIC SECRETIONS		REMARKS
			Free* acid	Total* acid	
		cc.			
Subject J. Residuum	1:55	19.0	45	52	
	2:05	11.0	60	72	
	2:15	7.5	35	62	
	2:25	7.3	40	57	
	2:35	5.5	52	67	
	2:45	5.0	60	77	
Ethylene from 2:55–3:01	2:55	9.0	45	70	
under 8 minutes	3:05	14.0	25	45	
	3:15	8.5	7	27	
	3:25	6.7	5	20	
	3:35	3.1	5	20	
	3:45	6.2	12	27	Smelling food
Subject E. Residuum	12:15	75.0	25	40	
	12:25	12.0	30	42	
	12:35	10.0	42	60	
	12:45	12.0	50	67	
	12:55	12.0	62	77	
	1:05	9.0	62	77	
Ethylene from 1:05–1:10	1:15	7.5	0	5	Some nausea
under 7 minutes	1:25	7.5	0	7	
	1:35	5.8	0	15	
	1:45	2.2	0	7	
	1:55	1.5	0	0	
Subject I. Residuum	1:00	35.0	37	50	
	1:10	8.2	45	62	
	1:20	13.0	50	67	
	1:30	10.0	55	70	
	1:40	11.0	50	70	
	1:50	10.8	45	62	
	2:00	10.5	50	65	
Ethylene from 2:10–2:15	2:10	11.1	47	60	
under 7 minutes	2:20	6.7	0	20	Bile
	2:30	4.0	15	30	
	2:40	8.5	27	45	

* Acidity is expressed in clinical units.

never abolished from the secretion and in two tests there was an increase in the amount of the secretion during the period of anesthesia and the period following. Typical results are shown on table 1.

Subject I frequently showed an increase in the amount of acidity of the secretion on smelling ethylene. This was probably a psychic secretion because this subject expressed a fondness for New Orleans molasses, which has an odor quite similar to that of ethylene.

The response to water stimulation under ethylene was studied in two subjects. One subject, J, normally failed to respond to water; subject I,

TABLE 2

Effect of ethylene anesthesia on the emptying of water from the stomach

Time of anesthesia—5 minutes

Amounts of water obtained from the stomach 20 minutes after drinking 500 cc.

SUBJECT J*		SUBJECT I†	
Control	Ethylene	Control	Ethylene
cc.	cc.	cc.	cc.
190	475	200	500
150	450	220	500
220	480	210	490

* Gastric glands of J. not stimulated by water.

† Gastric glands of I. stimulated by water. Ethylene depressed, but did not abolish the response.

TABLE 3

Effect of ethylene anesthesia on the emptying of a barium-milk meal from the stomach

Time of anesthesia—5 minutes

SUBJECTS	EMPTYING TIME		REMARKS
	Control	Ethylene	
Subject C	1 hr. 45 min.	2 hr. 45 min.	
Subject L	1 hr. 30 min.	2 hr. 30 min.	
Subject W	2 hr. 45 min.	3 hr. 30 min.	Nausea
Subject J	2 hr.	2 hr. 30 min.	
Subject S	2 hr. 30 min.	3 hr.	

Meal: 250 cc. barium milk and 20 grams malted milk.

Fluoroscopic examination from 5 to 10 minutes after the anesthesia showed an amotility of the stomach in all cases. In 2 cases (W and J) a broad transverse band of spasm, beginning at the incisura angularis and extending over the pyloric antrum so that it involved from one-half to three-fourths of the antrum producing an "hour-glass" effect, was observed.

whose gastric glands were normally stimulated by water, showed a response to water stimulation on being subjected to ethylene anesthesia for five minutes, but the response was definitely less than normal.

The gastric response to barium milk was followed in subject J. It was found that the appearance of free acid in the gastric contents was delayed from fifty to sixty minutes when ethylene was administered.

Motility of the stomach. We first determined the effect of the administration of ethylene for five minutes on the emptying of 500 cc. of water from the stomachs of two subjects. The water was drunk and the anesthesia induced. Twenty minutes after drinking the water, the stomach was emptied by tube. The results shown in table 2 demonstrate that ethylene delays the emptying of water from the stomach. With barium milk and fluoroscopic examination, we could not only determine the emptying time of the stomach, but we could also observe the effect of ethylene on the movements of the stomach. The results shown in table 3 demonstrate that ethylene anesthesia for five minutes delays the emptying of barium milk from the stomach, but relatively to a less extent than it delays the emptying of water.

When we examined the stomach fluoroscopically from five to ten minutes after the ethylene was withdrawn, we observed an amotility in every subject. In two subjects a transverse band of spasm, beginning at the incisura angularis and extending over the pyloric antrum so that it involved from one-half to three-fourths of the antrum producing an "hour-glass" effect, was observed. This condition of the stomach is known to occur during nausea and vomiting. One subject stated that he was nauseated, but vomiting did not occur; the other subject experienced no sensation of nausea, however.

Discussion. Since some of our subjects were medical students who had swallowed a stomach tube only one or two times previous to our experiments and who did not vomit the tube during recovery from the anesthetic, we are led to believe that ethylene affects only vomiting centers that are especially susceptible. We gave ethylene to two subjects who did vomit during recovery from the anesthetic. We found that one of these subjects had been told that he would vomit; and this subject told the second subject that he had vomited on coming out. So we believe that these two cases of vomiting were chiefly due to suggestion. (The results on these two subjects are not included in our paper, because of the occurrence of vomiting.)

Our results on gastric secretion show that ethylene definitely depresses gastric secretion. In this respect it is much superior to ether, which abolishes gastric secretion except in occasional instances following histamine or gastrin injections. We do not believe that this depression of gastric secretion is due to psychic influences, because all of our subjects were eager to take the anesthetic, had seen others take it and were not at all fearful of the outcome. Three of the subjects had taken the anesthetic several times before these experiments were performed and even looked forward with pleasure to the experience, because of previous ethylene anesthetic euphoria. We are inclined to believe that the depression of secretion was due either to stimulation of the gastric inhibitory center in the medulla, or to peripheral vaso-dilatation, or to a mild toxic effect on

the cells of the gastric glands. The observed variations in the amount of depression are not surprising in view of our knowledge of the variations which have occurred when other gastric secretory depressants have been studied.

The retardation of emptying of the stomach by ethylene might be due either to an amotility or to a pylorospasm. We observed amotility within the first ten minutes following the anesthesia, which shows that amotility is certainly a factor. We cannot say that pylorospasm was or was not present; but the fact that we observed spasm of the first part of the pyloric antrum in two cases suggests that pylorospasm is also a factor. Attention should be called to the fact that the disturbance of emptying of the stomach lasts longer than ten minutes as shown by our results on the emptying of water, almost no water being emptied from the stomach during the twenty-minute period. The fact that the emptying of the barium meal was delayed from thirty to sixty minutes by the ethylene anesthesia, shows that the stomach recovers in from thirty to sixty minutes after the anesthesia.

The results of our experiments show that ethylene can be only of limited value as an anesthetic for physiological experimentation on the stomach, and that the effect of ethylene anesthesia on gastric secretion and motility is opposite to the effect of physiological sleep and hypnosis.

CONCLUSIONS

1. Ethylene administered for a period of five minutes at the point of complete anesthesia depresses gastric secretion; but not to the extent that ether does, the secretion of some subjects being affected more than others.

2. Ethylene anesthesia delays the emptying of water and barium milk from the stomach. This delay is due to amotility and possibly to some pylorospasm.

Addendum: After completing the problem to our own satisfaction it was desired to have some x-ray pictures demonstrating photographically what we saw and traced on the fluroscope. We are indebted to Dr. H. P. Doub, Roentgenologist of the Henry Ford Hospital, for having made a series of roentgenograms on subject J. In this series a 500 cc. barium meal was used for the control and the ethylene tests. Comparison with the graphs in figure 1, subjects C, L, W, J and S, shows that the delay in emptying is equally as obvious as with the 250 cc. used in the barium meal tests on all these subjects. In this latter series on subject J, pressure on the stomach proved that it was difficult to express material into the duodenal cap (tried about five minutes after removal of the ethylene mask), but the manipulation was followed by peristaltic waves which however did not hasten the emptying, the total emptying time being 3 hours, 45 minutes. This observation demonstrates an increase in the tone of the pyloric sphincter.

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BODY-RIGHTING IN THE FOWL (GALLUS DOMESTICUS)

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It is our purpose to study the mechanics of flying in birds. As a preliminary step we decided to investigate the reflexes of body-righting and related phenomena in various birds. The fowl representing an intermediate type between the walking and flying birds, we chose as the first subject for investigation, and this paper is limited to the results obtained in this species.

In his monograph on "Körperstellung" Magnus (1924) summarizes the data, collected chiefly in his own laboratory, on the subject of body-righting. There is no adequate terminology in the English language for the numerous reflexes and we shall therefore indicate the German equivalents of the terms we used. In a general way, Magnus distinguished between static and stato-kinetic reflexes. The static reflexes he divided into two groups: *a*, the influence of the position of the head in space and as regards the body on the tonus of the limbs and that of the extrinsic eye muscles, and *b*, the so-called body-righting reflexes proper. The stato-kinetic reflexes are responses to the two varieties of movement of the body as a whole: translation and rotation. In his admirable treatise Magnus describes all these reflexes in great detail and for particulars the reader is referred to the original. We shall describe those of the Magnus and de Kleijn reflexes which we studied in the fowl. We might add that the body-righting in birds is not taken up in Magnus' book and that to our knowledge these reflexes have not been studied in the fowl.

EXPERIMENTAL. We employed a large number of common Leghorn and Rhode Island Red fowls, as well as a few fancy bred animals, such as Sebright Bantams. All the reflexes shown by the common fowl are much more vigorous in the Sebright Bantam and whereas any one of the latter will exhibit all the phenomena we are about to describe, this cannot be said of the common varieties of the fowl. As a general rule entirely normal animals were used in our tests, the experimental procedures involving no surgical work. In some cases, however, in the analysis of the origin of the various reflexes we performed unilateral or bilateral labyrinthectomy, or decerebration. Some of the reflexes were demonstrated before the Federation of the American Societies for Experimental Biology at the 1925 meeting at Cleveland.

STATIC REFLEXES. *a. Tonic labyrinthine reflexes on the extremities.* When the animal is held in the air in the normal position with the wings and legs free, the legs are extended and the toes are spread. Rotating the animal 180° around its longitudinal axis, legs uppermost, the legs and toes become flexed. These changes in the tonus of the limbs are just the reverse of what Magnus and his co-workers have observed in the mammal. But it is not possible to show that the labyrinths exert this effect on the tonus of the limbs and we are not certain that gravity plays no part in the attitude of the legs.

b. Tonic neck reflexes on the extremities and tail. When the animal is placed on a table on its back and the head is held in such a way that the beak points upward, the tail remains in line with the body and no differences can be observed in the attitude and tonicity of the two wings and the two legs. If now the left hand is lightly placed on the sternal surface of the animal (merely to prevent struggling) and by means of the other hand the animal's head is turned to one side, beak pointing to the right or to the left, then if the beak points to the animal's right, the right wing becomes markedly extended and the feathers spread, the right leg will be extended and the toes spread and the tail will be deviated to the right; at the same time the left wing is adducted and the left leg and toes are flexed. Exactly the reverse happens when the animal's head is turned so that the beak points to the left. It must be remembered that what appears to the casual observer to be the hen's lower leg is in reality its foot, since it walks on its toes. In speaking of the various changes of the tonus of the legs and toes, we shall call for convenience the foot from the ankle to the toes a part of the leg. Thus a flexion of the legs and toes means a flexion of the leg upon the thigh, the foot upon the leg and toes curled upon the foot. Bilateral labyrinth-extirpation has no effect upon this reflex, indicating that it depends entirely upon the proprioceptive impulses from the neck musculature. Lateral flexion of the head (Wendung) as differentiated from turning of the head (Drehung) has no effect upon the tonicity of the limbs and tail. Nor does dorsi- and ventriflexion of the head produce any effect. It seems that of the three types of neck reflexes described in mammals, the fowl shows only one, namely, that resulting from turning the head (torsion of the neck).

c. Compensatory eye position. As in the case of the other vertebrates, the fowl shows very definite deviation of the eyes when the head is slightly turned around the bitemporal or oral-occipital axis. Vertical and rotatory compensatory eye positions are assumed, and these positions are held as long as the abnormal posture of the head is maintained. The deviation is always in the direction opposite to that of the rotation, enabling the animal to preserve its visual field.

d. Tonic leg reflexes exerted upon the tail and head. When the animal is

held by the body in the air and the legs and feet and toes are passively extended, the legs parallel to the long axis of the body and the toes pointing straight back, the tail is turned practically vertically downward and the head is turned up. When the legs and feet are flexed, so that the toes touch the ventral surface of the animal, the tail turns up and the head turns down. The position of the body itself remains unchanged during the whole manipulation. This is very important, because tilting the body without changing the position of the legs will affect the head and tail, as will be shown later. This tonic leg reflex on the tail and head can be elicited much more easily in the Sebright Bantams than in ordinary fowls. In using these fancy breeds for the study of this reflex, we observed the following phenomenon: If the head is held immovably while the legs are extended or flexed, the tail will be turned down or up as usual. But, if the tail is held immovably, there is no effect on the head. It would appear, then, that the reflex is exerted primarily on the tail and on the head only indirectly through the deviation of the tail. This being a tonic reflex, the change of postures of the tail and head are maintained as long as the legs are extended or flexed. If the legs are extended backward causing a turning up of the head, and the head is forcibly bent down, then the head will immediately turn up again as soon as the pressure on it is released. Simultaneously with the tail and head response there is a slight flapping of the wings.

RIGHTING REFLEXES. *a. Labyrinthine reflexes on the head.* When the body of a fowl is turned through an angle around the longitudinal or transverse axis, the head retains its normal position, the beak pointing forward and slightly downward. When the animal is held in a side position, this reflex involves a twisting of the neck. Bilateral labyrinthectomy abolishes these reflexes and when the animal is held in a side position, the head hangs limply, the beak pointing downward and to the side, and when the animal is held back downward, the head falls back, the beak pointing straight downward.

b. Reflexes from body musculature on the head (Körperstellreflexe auf den Kopf). Magnus and his co-workers have found that after bilateral labyrinthectomy a rabbit's head which was hanging limply on one side, when the body was held in a side position in the air, suddenly assumes a normal position, when the lower side of the body comes in contact with the ground. They have experimental evidence that this righting influence on the head comes from unequal pressure on the general musculature of the two sides of the body, when the animal is resting on one side (Brettversuch). We could detect no such influence of the body musculature on the head in fowls with both labyrinths removed.

c. Reflexes from the body musculature on the body itself (Körperstellreflexe auf den Körper). This is another reflex discovered in Magnus' laboratory.

When a rabbit is put on the table on one side and the head held forcibly in the side position, the body will right itself and rest on all four feet, this involving the twisting of the neck. No such reflex exists in the fowl. There is some difficulty in the experimental procedure, however, since the wing covers the lateral aspect of the body, and when in the side position, the fowl is really resting on its wing and this makes the results somewhat doubtful.

d. Visual reflexes on the head. It is generally considered that animals with a practically monocular visual field (rabbit) have no visual righting reflexes on the head. This contention is based upon the fact that after bilateral labyrinthectomy such animals cannot keep their heads in a normal position, whereas higher mammals (dog, cat, monkey) can do so. It can, however, be shown that visual impulses play an important rôle in maintaining the normal posture of the head even in animals with a monocular visual field. We have examined a number of such animals but here we shall limit our remarks to the fowl. We have noticed this in studying the general effects of blindfolding of the fowl, which will be described later.

If a fowl is blindfolded and is kept in the air in the side position, the head gradually sinks, until it assumes a position which closely resembles that obtained in animals without labyrinths. In other words, the animal possessing labyrinthine righting reflexes of the head can *maintain* the normal posture of the head when the position of the body is abnormal only with the aid of visual impulses, at least shortly after blindfolding. At first we thought that the weight of the bandage used for blindfolding was responsible for the sinking of the head but later we merely put small pieces of adhesive tape over the animal's eyes and obtained similar results. We also tried unilateral blindfolding by covering one eye with adhesive tape. The results were not always uniform but in a general way the animals were capable of maintaining the head in an upright position when the body was held in a side position in such a way that the free eye was on top. But if the opposite was true, the head gradually sank into the side posture characteristic of an animal whose labyrinthine reflexes are lost. If an animal has its right eye covered with adhesive tape and held in the left side position in the air (left half of the body lowermost, right half uppermost), the head gradually sinks to the left, but if, while the animal's head is in this position the plaster is removed from the eye, the animal's head is immediately righted and maintained in a normal position.

e. Tilting reflexes. 1. *Forward and backward tilting:* By this we mean a rotation of the body through an angle about 30 to 45° from the normal on the horizontal axis passing transversely through the center of the body. The body is grasped either at the sternum or on the back between the wings and held suspended in the air, under these conditions the head, the tail and the extremities are all free to respond to the tilting. When the animal is

tilted so that the anterior portion of the body moves downward, the tail invariably turns up and its feathers become spread in a fan-like manner. If the animal is tilted so that the anterior portion of the body moves up, the tail moves down, becomes closely adducted to the body and its feathers are gathered together to the ventrum. There are no marked influences on the head, which under these conditions executes the ordinary symmetrical labyrinthine righting reflexes, no effect on the legs, but the wings sometimes execute a slight flapping movement (abduction followed by adduction). Since there is no effect on the legs, the tilting reflex may be elicited by holding the animal by the feet instead of by the body, as in this case the effects are much more marked.

Bilateral labyrinthectomy does not abolish this reflex. In working with animals without labyrinths, we observed the following phenomenon, which we shall designate as a paradoxical tilting reflex. If the animal is tilted backward, the tail is bent downward, as in a normal animal. But if the tilting progresses to such an extent that the head and neck of the animal, because of the lack of labyrinthine righting reflexes fall back of their own weight, closely resembling the opisthotonus position, the tail suddenly moves up. This paradoxical reaction probably depends upon the change in the degree of stretching of the anterior neck muscles, as it cannot be elicited by merely stretching the neck, but may be obtained even in normal animals, especially in fancy bred chickens, by forced dorsiflexion of the head and neck upon the back. The body may also be tilted on a transverse axis passing through the knees, by getting hold of the legs with both hands and allowing the body to tilt either forward or backward. One then observes that marked forward tilting causes the foot to be extended upon the leg and the toes to be extended and spread. Backward tilting, on the contrary, causes the foot to be ventroflexed upon the leg and toes curled.

2. *Lateral tilting.* When the body is turned through an angle of 45° from the normal on the horizontal axis passing longitudinally through the body, the corresponding wing becomes extended and abducted and the other wing flexed and adducted. At the same time the tail is laterally flexed and points in the direction of the extended wing. For instance, if the animal's body is tilted to the right, the right wing becomes extended and the tail turns to the right. This wing and tail reflex can be elicited, when the relation of the head to the body remains fixed, as well as after bilateral labyrinthectomy. Therefore, it does not depend upon the labyrinths, nor upon the neck musculature.

STATO-KINETIC REFLEXES. *a. Responses to translation.* When a fowl is grasped firmly by the back and moved suddenly forward and downward, the legs become markedly extended and the toes spread. At the same time there is a slight flapping of the wings. This reaction is of value to an ani-

mal in alighting from the air. It disappears after extirpation of the labyrinths. This landing and toe-spreading reflex was observed by Magnus in mammals (Sprungbereitschaft und Zehenspreitzung) and by Groebbels in birds (Landungsreaktion).

The other response to passive progressive movement observed by Magnus, that to sudden lowering or lifting of a board on which the animal stands, was never clearly defined in fowls we studied.

b. Responses to rotation. These may be divided into two types: responses to continuous rotation around an axis passing through the animal's body or around an axis of an observer, who holds the animal in his hands, with the arm stretched out to its full length. For studying the first type of response we placed the animal on a turn-table either in the normal position or lying on its back. With the animal standing when the turn-table is turned, during the rotation the head is deviated so that the beak points in the direction opposite to that in which the front part of the body is rotated. If the rotation is fast enough, there is also a deviation of the tail. The tail in this and in all subsequent responses described is always deviated in the same direction as the head (if deviated at all), so that the line from the head through the body to the tail is concave or convex, but never S-shaped. In the beginning of the rotation there is a slight flapping of the wings. When the animal is placed on the back and the table turned, there is a deviation of the head, so that the beak points in the direction of rotation of the front part of the animal. There is, however, no deviation of the tail upon rotation of the animal lying on its back. During the rotation there is also a nystagmus of the head, the deviation being the slow component of the nystagmus.

In the second type of rotation, around a vertical axis passing through the observer, the deviation of the head and tail will depend upon whether the long axis of the animal is continuous with the arm of the observer or at right angles to it. When the animal is held by the legs in the air in the normal position the long axis of its body continuous with the arm of the observer and together with it constituting the radius of curvature, then, if the animal's head is directed toward the observer, it is deviated in the direction of the rotation; if away from the observer, it is deviated in the direction opposite to that in which the animal is rotated (fig. 1). The tail is always deviated in the same direction as the head. If the animal is held by the legs in the air in the normal position, but with the long axis of the body at right angles to the observer's arm, which serves as the radius of curvature, the animal's head pointing to the observer's left, then turning the animal to the observer's right (tail foremost) causes a deviation of the head toward the observer or to the animal's left; turning the animal to the observer's left (animal's head foremost) causes a deviation of the head away from the observer or to the animal's right. In other words, no matter

what the direction of rotation, if the animal's head is foremost, the deviation is always away from the observer, and if the animal's tail is foremost, the deviation is always toward the observer, whether it is to the animal's right or left (fig. 2). As in other cases the deviation of the tail is always the same as that of the head, but whereas deviation of the head is always a component of nystagmus, we observed no nystagmus of the tail. Here, too, holding the animal's back downward and rotating it around the observer results in practically no deviation of the tail. If, while rotating the animal around himself, the observer holds the body of the animal with one hand and fixes the head with the other, there are produced deviations and nystagmus of the eyes instead of that of the head. We never observed

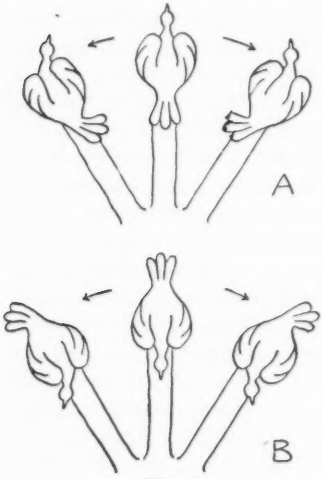

Fig. 1

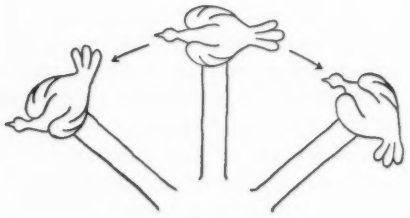

Fig. 2

Fig. 1. The effect of rotation upon head and tail. Fowl held in hand of observer in the normal position in the air, the long axis of the animal's body continuous with the arm of the observer and together with it constituting the radius of curvature. The animal rotated around the observer, arrows indicating direction of rotation. *A*, animal's beak pointing away from observer. Rotation results in a deviation of the head and tail in a direction opposite to that of rotation. B, animal's beak pointing toward observer. Rotation results in a deviation of the head and tail in the direction of rotation.

Fig. 2. The effect of rotation upon the head and tail. Fowl held in hand of observer in the normal position in the air, the long axis of the animal's body perpendicular to the observer's arm, which constitutes the radius of curvature. The animal rotated around the observer, arrows indicating direction of rotation. When rotated in such a way that the animal's beak points in the direction of rotation, the head and tail are deviated away from the observer. If animal's tail points in the direction of rotation, its head and tail are deviated toward the observer.

after-nystagmus of the head or eyes after the brief periods of rotation to which we subjected our animals. As was to be expected, nystagmus of the head and eye could not be elicited after bilateral labyrinthectomy.

Effects of blindfolding. If a fowl is blindfolded and placed on the floor it assumes a peculiar posture; legs are flexed, belly and head touching the ground. It seems to be prostrated. If examined for labyrinthine righting reflexes, it is found that they are fairly good with the animal in a normal position; present, but disappear when the animal is held with its back downward, head falling back as in a labyrinthectomized animal, and weakly positive with the animal in the side position, as already indicated above. The responses to tilting, forward and backward or lateral, are normal. The tonic neck reflexes are present. The animal responds to translation and rotation in the usual manner. When placed on its back it will make no attempt to turn over.

If allowed to remain blindfolded for a period of two or three hours, the animal gets up and stands on its feet. The long axis of the body is inclined forward, about 30° to the horizontal, beak touching the ground, tail directed upward. It makes no attempt to move, remaining just where it was placed. All the reflexes previously found to be present can be elicited, the labyrinthine righting reflexes on the head are slightly improved when the animal is in the side position, still absent, when the animal is held with its back downward. After twenty-four hours the posture of the animal seems to be quite normal, but it remains standing for hours in one place, without executing any progressive movements. All it does is occasionally turn its head or lift a foot. All reflexes are present except the labyrinthine righting reflexes, when the animal is in the dorsal position.

Righting from the dorsal position. It is a well-known fact that if a fowl is placed on its back on a table, it will turn over and regain its normal position. If it is placed very gently, it may remain in the dorsal position for several minutes (experimentum mirabile of Kircher); if put down forcibly while it struggles, it will right itself immediately. We were able to discern the following stages in the chain of reflexes responsible for this righting movement. In the first place the animal turns its head to one side, and as a result of the twisting of the neck, the tonic neck reflexes on the extremities cause the wing on the corresponding side to become extended and abducted and the leg to become extended and projected into the air. This causes the animal's body to fall over to the side of the extended wing, perhaps simply as a result of gravity. This brings the animal into a side position. Now the head is again turned, this time from the side to the normal position, and then the whole body is brought into the normal position, probably because of the neck righting reflexes described by Magnus in mammals. If, while the animal is lying on its back, its head is fixed with the beak pointing upward, either holding it firmly in the hand, or by

immobilizing it by means of a burette-clamp, the animal will remain lying on its back practically indefinitely. This may also be accomplished by putting the entire neck up to the head in a plaster cast. If powerful tactile stimuli are applied to the ventrum of the animal, under the above conditions, it will begin to struggle and may eventually turn over. But it is highly characteristic that it never turns over around the longitudinal axis of the body, the ordinary method of righting from the dorsal position. What it does is to powerfully extend its legs upward and forward until the toes are brought directly over the head, and then the entire body falls over forward and the animal lands on its feet, the beak touching the ventrum. This position being very awkward, the animal continues to struggle and the head has to be freed. Just as the animal will not change from the dorsal to the side position when the head is firmly immovable with the beak pointing upward, so will it not change from the lateral to the normal position when the head is held firmly with the beak pointing to the side (absence of body righting reflexes from the body musculature).

Effects of labyrinthectomy. We extirpated the labyrinths under chloroform anesthesia. We originally endeavored to remove all the three semicircular canals, but we found that the anterior vertical canal practically touches the cerebellum and it is impossible to remove it without injuring, or at least exposing, the cerebellum. We therefore left this canal intact, but destroyed its connection with the labyrinth. We did not examine the skulls histologically, but gross inspection at necropsy revealed a destruction of the labyrinth. We have performed a number of operations, either destroying one labyrinth, or both simultaneously, or first one and a few weeks later the other.

After unilateral labyrinthectomy, say on the right side, the animal shows a marked nystagmus of the head to the left, immediately upon recovery from the anesthesia. There is a nystagmus of the eyes in the same direction. Generally the nystagmus disappears and reappears continually, and it may be noticed that it disappears when the animal opens its eyes, lifts its head and fixes its gaze, and that it promptly reappears whenever the animal closes its eyes and allows its head to fall into a passive position. In less than an hour the fowl gets up and walks, but with a tabetic gait. In walking it has a tendency to fall to the right, and each time it falls the right wing becomes abducted and extended (lateral tilting reflex). The position of the head is normal, nystagmus can be obtained on rotation in either direction. In two or three days there appears a turning of the head of about 15 or 20° so that the left intact labyrinth is uppermost and the beak points to the left. The tabetic gait continues. When held in the air in the side position, right side of the body uppermost, the head is held in a normal position. When turned 180° on the long axis of the body, so the right side is directed downward, the head is held in an abnormal position,

the neck assuming the form of a semicircle and the beak pointing upward. When the animal is placed on its back, the beak still points to the left, the

Fig. 3

Fig. 4

Fig. 3. X-ray picture of a fowl unilaterally labyrinthectomized showing complete twisting of the neck. Animal is lying on its side, *C* indicating the crop, and its neck so twisted that the beak points backward.

Fig. 4. The position of the head in a fowl, after removal of both labyrinths, but at different times. *A*, animal after removal of right labyrinth. When held in the air the beak points to the animal's left. *B*, the *same* animal after removal of the left labyrinth. The neck is twisted in the opposite direction, the beak now pointing to the animal's right.

left leg and wing are extended, the right wing and leg are flexed (tonic neck reflexes on the extremities). Holding the fowl by the legs in the air,

the long axis of its body continuous with the arm of the observer, head pointing toward the observer, and rotating it around the observer as an axis, there is a marked nystagmus, when the animal is rotated to the observer's right, and a feeble nystagmus, when it is rotated to the observer's left. When placed on its back on the table, the animal turns over, but practically always towards the left. When put on the floor on its left side, the head assumes its normal position and the fowl rights itself immediately; on its right side, the head is twisted and the animal does not attempt to right itself (fig. 3). These changes are practically permanent except that with the passing of time the torticollis becomes more and more marked.

After bilateral labyrinthectomy the head is sometimes kept symmetrically, and sometimes has a tendency to fall over to one side (perhaps of its own weight) when the fowl is held in the air in the normal position. When held in the air in either side position, the head falls to the side, and when held back downward, the head falls passively back. It is clear that, as in case of the mammal, these righting reflexes on the head are of labyrinthine origin. Rotation of the animal produces no nystagmus, either of eyes or of the head. With the animal lying on the floor, loud sounds produced nearby fail to elicit any motor reaction, whereas powerful stamping on the floor results in a response. When placed on the floor on its back, the legs and wings remain symmetrical. Tonic neck reflexes on the extremities and tail do not differ from normal. All the tilting reflexes are present. When the animal is tilted backward and downward far enough, the head falls back of its own weight, and there is simultaneously produced a dorsiflexion of the tail, which we described above. When the animal is placed on one side of the table, the head is not brought into the normal position, indicating an absence of righting reflexes from the body musculature on the head.

If a second labyrinthectomy is performed a few weeks after the first, the results do not differ appreciably from those obtained after bilateral labyrinthectomy except that there is a temporary nystagmus after the second labyrinthectomy, like the Bechterew nystagmus, observed under similar conditions in mammals. But there is also a permanent twisting of the neck in the direction opposite to that of the twisting that follows the first labyrinthectomy (fig. 4).

The effect of neck posture on the direction of progression. One of our own operated animals held its head down and tail up at times, whereas at others it kept head and tail in the normal position. We noticed that when its head was down, in making an attempt to move it always walked backward. When its head was up it walked in the usual fashion. We then weighted the heads of some normal fowls, and although the head was kept down, they were still able to walk forward. We modified the procedure by

keeping the lower half of the neck ventroflexed by means of a gauze bandage tied around the neck and then tied around the trunk, bringing the two together. We found that under the above conditions the animals invariably walked backward. On removing the bandage, we observed that the animals immediately resumed their normal mode of progression. It appears that the condition of the ventral and dorsal neck muscles determines whether the animal shall be able to progress in a forward direction or not. In this connection it was thought that the backward movement might be due to a conscious attempt on the part of the animal to straighten its neck. We therefore decerebrated some animals, and by tactile stimulation made them execute a few steps. Here, too, ventroflexion of the head caused the animal to walk backward, although when not stimulated it remains quiet with the neck bent upon the chest. Incidentally, we investigated all the above described reflexes in such decerebrated fowls, and, as would be expected, found them all to be present.

We allowed some fowls held in the air in the supine position to fall from a height of about 3 meters, and we observed that they invariably landed on their feet. We had no opportunity to observe the course of events with the aid of moving pictures as employed by Magnus for the cat, but in so far as we could follow the progress of the animal with our eyes, we believe that the same chain of reflexes is involved here.

Body righting in chicks. We studied the various static and statokinetic reflexes and other phenomena related to body righting in chicks less than one week old. Practically all the reflexes we observed in the adult fowl are present in the chicks. We could not elicit the tonic neck reflexes on the extremities in any of the chicks examined. There is a mere suggestion of a tail in such chicks, but it always responded in various reactions involving tail movements. There was also a greater tendency to flapping of the wings as a general accompaniment of the reflex responses than in the adult. The response of the feet and toes, or to forward and backward tilting (around an axis passing through both knees) when the chicks were held by the legs was very pronounced. It almost looked like a mechanical effect of pulling on the tendons, but when anesthetized by means of chloroform and held in exactly the same position, the chick failed to respond to tilting by extending or flexing the feet and toes.

When the neck was kept ventroflexed by means of a bandage, the chicks invariably walked backward, just as the adult fowl did under similar conditions.

Discussion. There are a number of scattered data in the literature pertaining to tonic reflexes in birds, but practically all of them were obtained by experimenting on the pigeon. So far as we know, no work of this kind was ever done on the fowl. Groebbels made a systematic study of body righting and tonic reflexes, particularly in the pigeon, employing the

method developed by Magnus and his pupils. We have performed some experiments on pigeons, which will be reported in the future and we find that various reflexes in the pigeon are not the same as in the fowl. Since we know of no data on body righting and tonic reflexes in the fowl with which we could compare our own results, we shall be obliged to compare them with those obtained by Magnus on mammals and by Groebbels (1922) on pigeons. We shall refer to the work of these investigators as we discuss the various reflexes we observed in the fowl.

We obtained no clear-cut evidence of the presence or absence of tonic labyrinthine reflexes on the extremities. In mammals these reflexes are best observed when the preparation is in a state of decerebrate rigidity, as evidenced by decrease or increase of the tonus of the extensor muscles in accordance with the relative position of the otoliths in space. In birds, however, no decerebrate rigidity has ever been described, nor could we observe any in our own decerebration experiments, no matter where the section of the brain stem was made. We were therefore obliged to use normal, intact animals, and whereas we believe that in a fowl that is not struggling, the cerebrum has little influence upon the tonus of the extremities, we cannot affirm that fowl has no tonic labyrinthine reflexes on the extremities.

The situation is entirely different so far as the tonic neck reflexes are concerned. Here, too, we used normal animals, with the cerebrum intact and we obtained very definite results. These reflexes are very prominent in the adult fowl but are absent in the very young chick. Even though the wings of the bird have an entirely different function than the fore limbs of the mammal, they behave exactly the same way as regards the tonic neck reflexes, which would indicate that these reflexes are of a rather primitive type. It is noteworthy that in the fowl the tail shows these reflexes, becoming deviated towards the side of the extended wing and leg. It must be remembered that only the reflexes due to torsion (Drehung) of the neck are present in the fowl, the other two, those due to dorsi- and ventriflexion of the head and those due to lateral flexion (Wendung) being absent. From the historical standpoint it is interesting to note that in 1898 Verworn described a dissimilarity occasionally observed in the tonus of the legs of the fowl, when it is lying on its back and even has a photograph of a decerebrated fowl showing typical tonic neck reflexes on the leg and tail, but he failed to notice the connection between the posture of the head and neck and the tonus of the extremities.

There is another group of tonic reflexes, not described in mammals, reflexes from the legs exerted upon the tail and head. As already indicated the reflexes seem to be exerted primarily upon the tail and only indirectly upon the head, through the changed position of the tail. Similar reflexes were described by Groebbels for normal and by Baglioni and Matteucci (1909) and by Clementi (1910) for spinal pigeons.

As regards the righting reflexes the reflexes from the body musculature upon the head are absent. But we are not quite sure about the absence of righting reflexes from the body musculature exerted upon the body itself. In the great majority of cases, when a fowl was put on one side and the head kept in the side position, it failed to right itself, but in a few cases it did so after considerable struggle. It is our opinion that the righting reflexes from the body musculature upon the body itself do not exist in the fowl. The labyrinthine righting reflexes on the head, both symmetrical and asymmetrical, are very conspicuous in this species. A bilaterally labyrinthectomized animal is unable to keep its head upright and would therefore be classed by Magnus together with the guinea pig and rabbit as an animal without optic righting reflexes. But as we have shown, the visual impulses received by the fowl play an important rôle in maintaining the normal position of the head, when the body itself is brought into an abnormal position. Whereas the labyrinths are necessary to bring the head in a normal position, impulses coming from them are not sufficient to keep the head in that position, and this latter is accomplished through the action of visual impulses. In this connection it may be recalled that as shown by Pearcy and Koppányi (1925), in the goldfish, an animal having no neck, visual impulses coming in through a dislocated eye (the other eye being extirpated) are sufficient to tilt the body of the animal 45° in spite of the presence of the labyrinths.

The responses to tilting observed in the fowl do not differ from those described by Groebbels (1922) for the pigeon. They seem to be especially related to flying as a mode of progression, where the tail is employed as a rudder. From the detailed description we have given it is clear that the position of the tail depends upon the inclination of the long axis of the body to the horizontal. Groebbels did not use bilaterally labyrinthectomized pigeons, but on several fowls with both labyrinths removed, we observed perfectly normal responses to tilting. These responses, then, do not depend on the position of the otoliths, but it is difficult to tell just where the afferent impulses originate. The reflexes are equally well elicited whether the fowl is held by the legs or by the chest or by the belly or by the back between the wings, at the time the animal is tilted. It is our opinion that the reflex is elicited through a difference in the distribution of the weight of the body on the general musculature. The effect is probably produced through the stimulation of the proprioceptors in the muscles and joints. That changes in tonus of the muscles, even changes of a passive nature, can affect the position of the tail is shown by what we term a parodoxical tilting response.

The landing reflex (Sprungbereitschaft und Zehenspreitzung) is of labyrinthine origin as in the case of the mammals, since it disappears after bilateral labyrinthectomy. This reflex was observed by Groebbels in

the normal pigeon, but he did not attempt to localize the origin of the stimulus. The reader will recall that the position of the legs which the animal assumes in landing leads to the dorsiflexion of the tail, this being one of tonic reflexes from the limbs on the tail described above. It is clear that this position of the tail is very helpful as a rudder in directing the body of the animal forward and downward. On the other hand, in flying in a horizontal plane the legs become continuous with the long axis of the body and this calls forth a ventriflexion of the tail, the other tonic reflex from the legs upon the tail. This gives the whole body of the animal, including the leg and the tail, a "stream line" form, offering the least resistance to the air.

As regards the responses to rotation, the deviations are generally in the same direction as in the case of the mammals. But in the fowl it is much more easy to elicit and to observe nystagmus of the head than that of the eyes. There is scarcely any indication of an after-nystagmus. Although the deviation of the tail is always to the same side of the animal as that of the head, we were never able to observe any nystagmus of the tail.

The most striking immediate effect of blindfolding is the peculiar crouching posture assumed by the animal and the almost complete absence of spontaneous movements. This may be looked upon as an inhibition of voluntary activity due to the absence of visual impulses. But it is hard to conceive of a lack of stimulation acting as an inhibiting agent. What seems more probable is that visual impulses are necessary for the initiation of movement under ordinary conditions. This may well account for the phenomenon known as experimentum mirabile. As originally described by Kircher, this experiment involved a fixing of the eyes of the bird upon a chalk-line drawn upon the floor, on which the animal was placed. Later on this was found to be unnecessary as the animal maintained the mirabile position as long as it did not move its head, thereby changing its visual field. We have been able to keep the fowl in the "mirabile" position for a very long time merely by holding or fixing its head, or by blindfolding. Incidentally we found that the "turning over" or righting the body from a supine position also depended upon an initial turning of the head and twisting of the neck, by which the tonic neck reflexes upon the extremities are elicited. No animals in our experience ever turned over in the usual manner, i.e., around the long axis of the body, when in the supine position with the head fixed, beak pointing upward. Incidentally, too, we found under these conditions, if stimulated by powerful tactile stimuli, the animal will turn over in a somersault, heels over head, a method never employed when free.

There is very little to say about the effects of labyrinthectomy. The results do not differ from those observed by previous investigators working on various species. A peculiar phenomenon, however, showed itself

in the head nystagmus following unilateral labyrinthectomy, which disappeared when the animal kept its eyes open, and reappeared when it closed them. It seems that through visual impulses (by fixing its gaze upon an object) the animal is able to inhibit the nystagmus reflex.

Several of the older investigators, among them Magendie, Flourens and Ewald (1892), observed backward walking of birds after intracranial injuries, but none of them could produce this phenomenon at will. We noticed backward walking in fowl under similar circumstances, but we also found the cause of it, namely, ventriflexion of the neck. We also saw it in blindfolded fowls, when for one reason or another, their neck became ventroflexed. We were able to call forth backward walking at will by keeping the neck bent by means of a bandage, and to abolish it and restore the normal mode of progression by removing the bandage.

In conclusion we wish to state that the experiments reported are preliminary to a study of the reflex mechanism involved in flying. Orderly but aimless flying can be carried on even by decapitated birds, as was shown by Tarchanoff (1884). But orderly and purposeful flying is a complex of chain reflexes varying according to the manner and the direction of progression. Many of the static and stato-kinetic reflexes described above are directly related to the taking to the air and alighting, flying up and down as well as in a horizontal plane, and we expect to further analyze the various modes of progression in the air by means of "slow motion" moving pictures.

SUMMARY

We have observed a number of static and stato-kinetic reflexes in the fowl, previously described in various mammals and birds, as well as the following hitherto undescribed phenomena.

1. There are in the fowl tonic neck reflexes exerted upon the wings and tail (neck → wings, and neck → tail reflexes).

2. There exist tonic leg reflexes exerted upon the tail and head (leg → tail, and leg → tail → head reflexes).

3. Visual impulses play an important rôle in the maintenance of the posture of the head, when an intact fowl is placed in an abnormal position.

4. There is a paradoxical dorsiflexion of the tail when the head and neck are forcibly bent backward, or when the head of a bilaterally labyrinthectomized fowl is allowed to fall backward.

5. Tilting the animal on an axis passing through the knees causes a change in the tonus of the muscles of the leg and toes.

6. Head nystagmus induced through unilateral labyrinthectomy in the fowl disappears when the eyes are open, and reappears when the eyes are closed.

7. The fowl is unable to turn over from the supine position if its head is held or fixed immovably, beak pointing upward.

8. Backward walking can be induced by ventroflexion of the neck.

9. All the reflexes described can be observed in decerebrated fowls.

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STUDIES ON METABOLISM

IV. The Basal Metabolic Rate of Normal Dogs

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The expense involved in the construction of a respiratory calorimeter and the cumbersomeness of the technique have, until recently, exerted a marked influence in limiting investigation relative to the basal metabolism of dogs. The method described by one of us (Kunde, 1922–1923) has greatly reduced the technical and economic difficulties regarding such research. With the development of new lines of investigation pertaining to the heat production of dogs, it seems evident that more data regarding the basal metabolism of normal dogs are necessary. Furthermore, a comparison of the data obtained by different investigators should be made, even though the number of dogs in each investigator's series is relatively small.

This report presents the results of a first attempt to compare the available data of the basal metabolism of normal dogs, where the observations have been made by several investigators employing modern methods but quite different technique. A description is also given of the regime adopted in Hull physiological laboratory for the care of dogs (unless otherwise stated) in the metabolism series. Briefly—the dogs are not confined to cages but live in separate roomy booths with ample space for limited activity. In addition to this they are allowed to exercise vigorously with other dogs for 2 to 3 hours daily in a large room. These routine measures are taken to keep the dogs in normal condition with respect to muscle tonus and are designated "the standard amount of activity."

The metabolism tests are made early in the morning. Each dog lies quietly on a table for at least 45 minutes immediately preceding the test. The dogs are fed once daily at least 18 to 20 hours before making a metabolism test. The routine diet (for a 10 kilo dog) consists of a high protein maintenance requirement of approximately 200 grams of hashed raw lean meat, 50 grams bread and 250 cc. of whole milk, thoroughly mixed together before giving it to the dog. Occasionally they are given a cooked bone.

[1] This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

TABLE 1

Showing the basal metabolic rate of 32 normal dogs

NAME OF INVESTIGATOR, NUMBER OF DOG	INTERVAL OF TIME DURING WHICH TESTS WERE MADE	NUMBER OF TESTS ON EACH DOG	AVERAGE NUMBER OF CALORIES OF HEAT PRODUCED PER 24 HOURS PER DOG	GREATEST DEVIATION OF A SINGLE TEST FROM THE AVERAGE IN TERMS OF PER CENT FOR THIS DOG	AVERAGE WEIGHT IN KILOS	SURFACE AREA	AVERAGE NUMBER OF CALORIES OF HEAT PER SQUARE METER PER 24 HOURS
*Rubner**	June 10–	10	273.39	+15.8	5.12	0.3327	819.8
Dog F1	July 12			−15.9			
	July 8–11	4	289.35	+9.3	5.29	0.3400	850.8
				−6.2			
	July 11, 12	2	230.7	+0.42			
				−0.42	4.545	0.3073	750.7
Dog (♂)							
Table 3		2	608.9	±2.7	11.01	0.5546	1,097.0
Dog (♂)	1882, Jan 28–	4	471.5	+1.1			
Table 10	Feb. 3			−6.1	6.66	0.3965	1,190.0
Dog (♂)	1882	4	744.61	+3.7	20.31	0.8337	893.3
Table 108	June			−3.4			
Dog (♂)	1884			+1.07			
Table 108	June	4	931.3	−1.09	25.5	0.9703	959.6
Dog (♂)	1883			+2.4			
Table 108	June	3	946.1	−1.4	23.53	0.9198	1,028.0
Dog (♂)	1885			+3.4			
Table 108	March	4	936.3	−0.92	24.50	0.9448	991.0
Dog (♂)	1885			+9.8	26.2	0.9879	1,003.0
Table 108	May	4	991.1	−8.9			
Lusk†	1911		535.0		15.8	0.705	759.0
♀ I							
♀ II	1912		389.0		9.3	0.500	785.0
♀ III	1914		401.0		12.5	0.603	664.0
♀ IV	1914		485.0		12.7	0.610	795.0
♀ XIV	1914		516.0		13.1	0.622	829.0
	1915		532.0		14.6	0.669	814.0

TABLE 1—*Continued*

NAME OF INVESTIGATOR, NUMBER OF DOG	INTERVAL OF TIME DURING WHICH TESTS WERE MADE	NUMBER OF TESTS ON EACH DOG	AVERAGE NUMBER OF CALORIES OF HEAT PRODUCED PER 24 HOURS PER DOG	GREATEST DEVIATION OF A SINGLE TEST FROM THE AVERAGE IN TERMS OF PER CENT FOR THIS DOG	AVERAGE WEIGHT IN KILOS	SURFACE AREA	AVERAGE NUMBER OF CALORIES OF HEAT PER SQUARE METER PER 24 HOURS
♀ XV	1917		413.0		9.1	0.488	845.0
♀ XVI	1917		480.0		10.6	0.540	888.0
♀ XVII	1918		504.0		14.9	0.678	743.0
♀ XVIII	1919–1921		398.0		10.8	0.547	728.0
♀ XIX	1920		422.0		9.9	0.516	818.0
	1921		420.0		9.4	0.499	842.0
	1923–1924		396.0		11.5	0.571	694.0
♀ XX	1921		358.0		9.1	0.488	734.0
Kunde‡	1921	7	360.1	+1.0	10.2	0.526	686.0
Dog (♀) I	March. 21–Apr. 8			−1.6			
(♂) II	1921, July 29–Sept. 17	42	465.3	+10.0			
				−9.8	12.41	0.600	775.0
(♀) IV	1923, June 16–27	11	437.5	+2.0	11.2	0.560	781.0
				−3.3			
(♂) V	1926 Jan. 13–Feb. 22	10	433.6	+2.3	11.1	0.557	777.3
				−1.6			
(♂) VI	1923 May 16–June 3	17	447.0	+5.8	10.2	0.526	843.0
				−6.0			
(♂) VII	1926, Feb. 26–Mar. 16	10	425.3	+4.9	10.3	0.530	802.4
				−5.3			
(♀) VIII	1926, Mar. 5–22	10	544.0	+4.4	15.4	0.693	778.4
				−5.5			
Steinhaus	1925, Mar. 5–28,	37	393.5	+8	8.75	0.4755	827.5
Dog (♂) 1	Apr. 26–May 22			−9.8			

TABLE 1—*Concluded*

NAME OF INVESTIGATOR, NUMBER OF DOG	INTERVAL OF TIME DURING WHICH TESTS WERE MADE	NUMBER OF TESTS ON EACH DOG	AVERAGE NUMBER OF CALORIES OF HEAT PRODUCED PER 24 HOURS PER DOG	GREATEST DEVIATION OF A SINGLE TEST FROM THE AVERAGE IN TERMS OF PER CENT FOR THIS DOG	AVERAGE WEIGHT IN KILOS	SURFACE AREA	AVERAGE NUMBER OF CALORIES OF HEAT PER SQUARE METER PER 24 HOURS
(♂) 2	Feb. 22–May 22, 1925 Dec. 28, 1925 Feb. 1, 1926	80	578.9	+10.7 −11.6	16.17	0.7168	808.3
(♂) 4	1925 Oct. 4–31	23	579.5	+11.0 −14.0	17.51	0.7551	772.3
(♂) 5	Dec. 2, 1925 Feb. 9, 1926	43	571.4	+6.7 −11.4	20.29	0.8332	685.8
(♂) 6	Sept. 14, 1925 Feb. 27, 1926	35	698.1	+3.4 −10.3	26.83	1.003	695.5
(♂) 7	July 14, 1925 Feb. 6, 1926	48	661.8	+8.4 −10.09	21.13	0.8559	773.1
Boothby§ F-257 (♀)	1922 Mar. 18–July 25	69	650.4	+17 (Av. Dev. 4.8)	17.4	0.752	864.8
F-754 (♀)	1922, Oct. 12–Nov. 14	18	508.8	+6.0 −13.0 (Av. Dev. 3.7)	12.18**	0.5929	858.9
F-513 (♂)	1922 Oct. 4–Nov. 17	48	530.4	+7.0 (Av. Dev. 3.2)	12.63**	0.6074	873.2
F-5 (♀)	Dec 3, 1922 Jan. 26, 1923	14	777.6	Greatest var. not given (Av. Dev. 5.9)	14.44**	0.6642	1,171.0

* Rubner (1902): Loc. cit., pp. 52, 59, 292.

† Lusk: Loc. cit., p. 214.

‡ Kunde: Loc. cit., (1923), data for dog I, p. 416, II, p. 424. Dog III is not included in this table of normal dogs because it was found that she was in a pathological condition at the time the tests were made.

§ Boothby and Sandiford: Loc. cit., pp. 107–119.

** Weight averages calculated by the authors.

Our records over a period of 6 years show that dogs fed on such a diet remain entirely free from symptoms of nutritional disturbances and may not vary more than 0.2 to 0.3 kilo in weight over many months.

Mention should be made here of two infectious diseases to which dogs are susceptive and which seriously upset results if experiments are anticipated which extend over long periods of time. These diseases are mange and an acute respiratory infection commonly called snuffles. Our dogs are exposed to snuffles as soon as they enter the laboratory. One attack confers immunity. This is not so of mange. Dogs may have frequent and repeated attacks of mange. If allowed to progress the condition becomes serious and obstinate to treatment. It is accompanied by increase in body temperature, intense itching, skin lesions, loss of hair, increase in basal metabolism, loss of weight, etc. If discovered the first day or two of its onset it is very amenable to treatment and becomes a transient condition of no consequence. All dogs are kept in the laboratory at least two months preliminary to actual experimentation. During this time they receive the training necessary to make them fitting subjects for basal metabolism experiments. The nutritional plane is established. The maintenance diet is determined, immunity to snuffles conferred and treatment for mange given if necessary.

The basal metabolic rate of normal dogs. Table 1 contains the individual average of the data obtained from studying the basal metabolism of 32 normal dogs. These results are expressed in terms of calories of heat produced per square meter of body surface per 24 hours. The surface area is determined from the formula $\sqrt[3]{W^2} \times 0.112$. Three types of apparatus have been employed in making these observations. First, the *calorimeter* used by Rubner (1891; 1902) in making observations on 4 dogs and by Lusk and Dubois (1924) for studying 11 dogs. Williams (1912) published a detailed description of the calorimeter in Lusk's laboratory. This is the first paper in Lusk's series on *Animal Calorimetry.* Second, *a muzzle connecting trained dogs to a closed respiratory system.* Kunde (1922–1923) and Steinhaus, each working independently, have used this method in studying 13 dogs. Third, *a head piece connecting dogs to an open circuit, Tissot type of apparatus,* employed by Boothby and Sandiford (1923) in making observations on 4 dogs of their series. A graphic presentation of the results is contained in figure 1. In this figure each investigator's results are collected in a separate group. The arrangement of the groups conforms to the chronological publication of the respective methods used.

The conditions necessary in determining the basal metabolism of dogs according to these methods are quite different, but need not be discussed here, in as much as each author has given a description of the technique involved upon the publication of his method. Table 2 gives a comparison

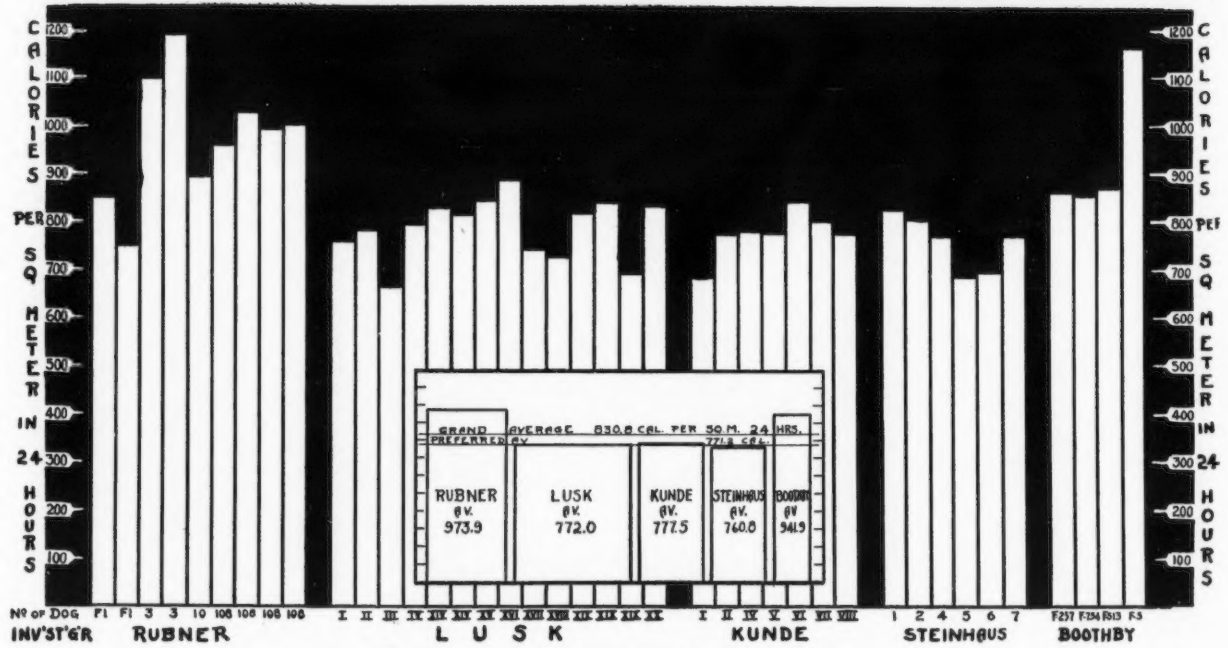

Chart 1. Comparing averages of individual dogs

(Insert). Comparing individual investigator's averages with grand and preferred averages

of the average basal metabolic rate of normal dogs as determined by the several observers. These results are calculated by taking the averages of all dogs in each investigator's series as given in table 1. The serial average obtained by Rubner is 973 and by Boothby and Sandiford, 941.97. These averages are much higher than the serial averages of 772 obtained by Lusk, 777.5 obtained by Kunde and 760.8 obtained by Steinhaus.

It is difficult to explain the high metabolism reported by Rubner. A review of his inspiring work gives conclusive evidence that the stimulating

TABLE 2

Showing average basal metabolism of each investigator's entire series of dogs: variations in each series and deviations of serial averages from the grand average and from the preferred average

NAME OF INVESTIGATOR	NUMBER OF DOGS STUDIED	AVERAGE NUMBER OF CALORIES OF HEAT PER 24 HOURS PER SQUARE METER OF BODY SURFACE OBTAINED BY EACH INVESTIGATOR FOR HIS TOTAL NUMBER OF DOGS	GREATEST DEVIATION OF THE AVERAGE RESULTS OF ANY DOG IN A SERIES FROM ITS SERIAL AVERAGE	DEVIATION OF INVESTIGATOR'S SERIAL AVERAGE FROM THE GRAND AVERAGE OF 820.8	DEVIATION OF INVESTIGATOR'S SERIAL AVERAGE FROM THE PREFERRED AVERAGE OF 771.2
Rubner	4	973.0	+22 −23	+14.6	+20.8
Lusk and DuBois	11	772.0	+15 −14	−8.9	0
Kunde	7	777.5	+8 −9	−6.9	+0.8
Steinhaus	6	760.8	+9 −10	−9.2	−1.3
Boothby and Sandiford	4	941.97	+24 −9	+10.7	+18.0

effect of the foodstuffs was well controlled. It is quite possible that the increased metabolism may have been due to uncontrolled activity of the dogs while in the calorimeter. It should be remembered here that in most instances Rubner's dogs remained in the calorimeter 24 hours and our knowledge of the influence of slight amounts of activity on the basal metabolism has greatly increased since these observations were made by Rubner. The high metabolic rates reported by Boothby and Sandiford are probably due to the fact that the dogs have not received a sufficient amount of training to enable them to lie perfectly relaxed during the metabolism tests.

Since the averages obtained by Rubner and by Boothby and Sandiford are entirely out of keeping with the other averages, it seems best not to include them in a grand average which should serve as a future guide in comparing the results obtained from basal metabolism studies of normal dogs. Consequently we have arranged two final averages. The first, called the grand average, is obtained by averaging the results of the 32 dogs collected in table 1. According to this the heat production per square meter of body surface of normal dogs per 24 hours is 830.8 calories. This we believe is a false average raised above the true level by the high results obtained by Rubner and by Boothby and Sandiford. It should not be used as a guide in comparing general averages. The second is called the *preferred average*. It includes the average results obtained from the 24 dogs observed by Lusk, by Kunde and by Steinhaus, but excludes the averages reported by Rubner and by Boothby and Sandiford. According to this, the average basal metabolism of 24 normal dogs is 771.2 calories of heat per square meter of body surface per 24 hours. The 13 dogs studied by the authors are within 10 per cent ± of that figure. Chart 1 (insert) graphically compares each investigator's serial average with the grand and preferred averages.

Our purpose in presenting these results is not to proclaim that the heat production in dogs per square meter of body surface (as determined by Meeh's formula) is constant. Indeed forthcoming publications of this series present evidence showing that in dogs, as with other animals, the heat production per square meter of body surface changes with the nutritional plane, the amount of physical activity and other conditions less easily explained. Benedict (1919) has discussed some of these factors in detail. However, our results seem to indicate that if the amount of physical activity, the maintenance requirements and protein intake of dogs are properly taken care of, the average results of ten or more short period tests (15 minutes) will fall within 10 per cent ± 771.2 calories of heat per square meter per 24 hours.

Meeh's formula with a constant of 0.112 has been used in determining the surface area of these 32 dogs. This, of course is subject to much criticism inasmuch as no dog has a form typically representative of the varying configurations found in the species canis. An attempt has been made by the senior author to keep the relative values for the surface area of the several dogs comparable by choosing dogs of approximately the same weight, but even then striking variations in form are apparent.

The important issue in the experimental laboratory, however, is not so much to be able to closely check the results obtained on some other dog, as to *accurately* determine the normal basal metabolism of the animal under experimentation. The normal for that animal is the base line from which the experimental results in question are calculated.

SUMMARY

1. The basal metabolism of 32 normal dogs has been tabulated. Three types of apparatus have been used in making these determinations.

2. The average heat production of normal dogs per square meter of body surface per 24 hours as determined by Rubner is 973 calories; by Lusk and Dubois, 772 calories; by Kunde, 777.5 calories; by Steinhaus, 760.8 calories and by Boothby and Sandiford, 941.97 calories.

3. The preferred average including the results obtained from the 24 dogs studied by Lusk and Dubois, by Kunde and by Steinhaus suggests that the average basal metabolism of normal dogs is 771.2 calories per square meter per 24 hours. The 13 dogs studied by Kunde and by Steinhaus fall within 10 per cent ± of this.

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FURTHER OBSERVATIONS ON THE ABSORPTION OF UNDIGESTED PROTEIN

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In a previous paper (Hettwer and Kriz, 1925) on the absorption of undigested protein from the alimentary tract of guinea pigs as determined by the direct anaphylaxis test, the observations described included the following which are of significance in connection with the present investigation. Guinea pigs sensitized three weeks previously to horse serum showed no signs of anaphylactic shock upon the direct intestinal injection of small amounts of horse serum (1.0 to 2.0 cc.); but showed the usual symptoms after *a*, the direct injection of small amounts into a ligated loop of small intestine; *b*, the direct injection into the unligated intestine of very large amounts (10.0 to 15.0 cc.).

These observations led to the assumption that an unusual increase of intra-intestinal pressure as a result of artificial stasis, is an important factor in the absorption of undigested protein from the intestine under the given conditions. Obviously, a more detailed and perhaps more convincing demonstration of this factor of intra-intestinal pressure was desirable. We believe that the experiments now to be reported provide a more adequate foundation for our assumption and in addition furnish ground for believing that minute amounts of protein are normally absorbed from the intestinal tract in a whole or undigested form.

METHODS. As in our previous investigation, the direct anaphylaxis test was employed to determine the absorption of undigested protein; that is, the experimental animal (guinea pig) was at the same time the test animal having been previously sensitized to the particular protein (horse serum). To ensure uniformity in the animals used it was considered advisable always to employ animals of nearly the same age (three months) which had been sensitized a definite number of days previously (twenty-one) with the same sensitizing dose (0.01 cc. horse serum) via the same route (intra-peritoneal).

In the animals manifesting anaphylactic shock the progression of symptoms was remarkably constant, appearing in an interval of thirty to sixty minutes after the intoxicating dose was administered. There was hyper-

activity, sniffing, wiggling of the ears and rapid chewing movements, pawing at the mouth, frequently urination and defecation but never complete paralysis and death. Apparently it is almost impossible to give an intoxicating dose via the intestinal tract large enough or at least to force an absorption of the serum into the circulation rapidly enough to produce the severest symptoms of complete paralysis and death. The animals not manifesting symptoms of anaphylactic shock after administration of a toxic dose were invariably tested for sensitization after an interval of two hours by an intra-peritoneal injection of 2.0 cc. horse serum. Distinctly positive signs of shock by this test, therefore, indicated that a previous negative result of intestinal injection was not due to a failure of proper sensitization of the animal.

The following *a priori* considerations of the factors involved in the study provided the basis for our experimental procedure. Given the fact demonstrated in our first experiment that anaphylactic shock results from the injection of a small amount of protein into a ligated loop of small intestine and not from the injection of a like amount into the unligated intestine, the obvious possible differences in the two experimental conditions are that in the unligated intestine there is a large absorbing surface and unaltered opportunity for proteolytic digestion, there is little or no stasis and consequently but little intra-intestinal pressure developed; whereas in the ligated loop there is a smaller absorbing surface, less digestive juices present and above all there is a stasis with the result of longer and more intimate contact of the contents with the same absorbing surface and increased intraintestinal pressure due to the effect of peristalsis, weight of the intestine and breathing on the confined loop contents.

Our previous work has, however, shown that the presence or absence of digestive juices, that is, such as can be readily washed out of a loop, made no difference in the result. Consequently we are left with the three factors of extent of absorbing surface, longer and more intimate contact with the same and increased intra-intestinal pressure in the ligated loop. The extent of absorbing surface will vary directly with the length of loop used if the diameter of the intestine is constant, that is, fairly uniform in all the animals used. The intra-intestinal pressure, on the other hand, will vary inversely with the length of loop, other things being equal; and it will vary directly with the volume content of the loop which in our experiments would mean the volume of fluid or the size of dose injected plus whatever fluid may be secreted into the loop. The amount of fluid secreted into the loop we found to be fairly constant for a certain length of loop and a certain length of time. The intimacy of contact of the contents with the absorbing surface may be taken to be a consequence and to vary directly with the intra-intestinal pressure. The duration of contact, however, is uncontrollable in our procedure in as much as we are forced to wait for the

anaphylactic symptoms. It was usually from thirty to sixty minutes. Thus the remaining factors are absorbing surface and intra-intestinal pressure both of which can be readily controlled by the length of the loop and the size of the dose used.

In the following four series of experiments these factors were alternately varied and kept constant. No account was taken in these first series of possible variation in the diameter or distensibility of the intestine of the respective test animals. Individual differences in these respects do exist, of course, and are of some importance as is brought out in later experiments. They are, however, not so great as to vitiate the results of the first series. Each series was performed in duplicate to indicate the constancy of results.

RESULTS. *Series A. Constant dose with varying length of loop.* A 2-cm. median incision was made in the abdomen of an anesthetized guinea pig and a loop of fairly empty small intestine drawn forth. A ligature

TABLE I

NUMBER	LENGTH OF LOOP	INTOXICATING DOSE	ANAPHYLACTIC SYMPTOMS	
			Primary series	Duplicate series
	cm.	*cc.*		
1	Unligated	1.3	−	−
2	20	1.3	−	−
3	15	1.3	−	−
4	12	1.3	+	−
5	10	1.3	+	+
6	7	1.3	+	+
7	5	1.3	+	+

was tied at one point and a given distance from it (measured by placing a thread of the required length along the outer curvature of the gut) another ligature was tied. Very close to one of the ligatures, 1.3 cc. horse serum were injected into the loop by means of a fine needle and syringe. Before withdrawing the needle, a third ligature was placed just beyond it in order to isolate the needle hole, thereby preventing possible leakage of the serum into the abdominal cavity. On withdrawing the needle a bit of cotton was temporarily held over the hole to absorb any minute amount of serum that might ooze from it. The loop was then replaced into the abdomen, the incision sutured and the animal allowed to come out of the anesthetic. After this the animal was kept under observation for about two hours, particular attention being directed to the appearance of signs of anaphylactic shock.

The above procedure was carried out on two series of seven guinea pigs each, using the constant dose of 1.3 cc. horse serum and decreasing lengths

of loop from 20 to 5 cm., with an extreme loop length represented by the unligated intestine. The results are shown in table 1.

It will be noted that in both the primary and duplicate series the symptoms of anaphylactic shock were invariably manifested by the animals in which the shorter lengths of loop were employed and not by those in which the longer lengths were used, including under long lengths also the unligated intestine. Between the extremes of long and short lengths the primary and duplicate series differ somewhat from each other; sometimes positive and sometimes negative symptoms obtained.

The factors to be considered in the present series are extent of absorbing surface and intra-intestinal pressure which vary in opposite directions with the length of the loop. Taking extent of absorbing surface first, the results show that positive symptoms of shock are not obtained when the loop is long, that is, when the absorbing surface is large; but only when the loop is short, that is, when the absorbing surface is relatively small and the intra-intestinal pressure very high do the symptoms of shock appear. The inference must be that the extent of absorbing surface is of little consequence in the absorption of undigested protein. On the other hand, if intra-intestinal pressure is the factor of importance, we should expect the most invariably positive results with the shorter loops in which the pressure is high and invariably negative results with sufficiently long loops. This expectation is exactly borne out in the present series.

The variable results obtained between the extremely short and long loops were attributed to individual differences between guinea pigs in the diameter and distensibility of the intestine and these differences formed the subject of separate experiments reported in series E. It is recognized, of course, that the actual intra-intestinal pressure developed depends not only on the length of loop and differences in the diameter and distensibility of the gut but also on the small, variable amount of original intestinal contents which may have been present and on the fluid secreted into the loop after it is replaced into the abdomen. But since loops nearly devoid of contents were chosen, this factor may be considered as almost negligible. On the other hand, fluid secreted into the loop is of some importance in raising the intra-intestinal pressure. It was part of the routine procedure to kill the guinea pig after two hours, open the abdomen and examine the loop used. In nearly every case the particular loop and that one only appeared more distended and the blood vessels of that region were dilated. The loop was then cut out and the contents measured in a burette. The usual finding was that in addition to the horse serum injected there was about one cubic centimeter of a watery or slightly viscid fluid for a 15 cm. loop, more or less according to the length of the loop. Even the unligated intestine appeared to be well filled but not greatly distended with a watery secretion. This fluid secretion is, no doubt, due to the mechanical stimula-

tion unavoidably produced in handling the intestine and not to a specific stimulation by the horse serum because it is found even in unsensitized guinea pigs similarly injected with normal saline.

In a ligated loop this secretion must help to raise the intra-intestinal pressure considerably just as breathing, the weight of the intestine and peristalsis tend to raise it. But since the amount secreted is quite constant and directly proportional to the length of the loop, this fact of fluid secretion into the intestine does not modify the inference based on the results of this first series, namely, that unusually increased intra-intestinal pressure is the factor of importance in the absorption of undigested protein. The influences mentioned represent the uncontrolled and largely uncontrollable factors in our experiments. It should be conceded, however, that their effect could only be determinative of positive or negative results in the middle portion of the series.

Series B. Constant length of loop with varying dose. The method of procedure in this series was exactly the same as for series A, except that a

TABLE 2

NUMBER	LENGTH OF LOOP	INTOXICATING DOSE	ANAPHYLATIC SYMPTOMS	
			Primary series	Duplicate series
	cm.	cc.		
1	15	0.4	−	−
2	15	1.0	−	−
3	15	1.3	−	+
4	15	2.0	+	+
5	15	3.0	+	+
6	15	4.0	+	+

constant, convenient length of loop was taken, namely, 15 cm., and a different dose of horse serum injected at each trial. The series comprised six guinea pigs and was performed in duplicate. The dose of horse serum varied between the limits of 0.50 and 4.0 cc.

The results appearing in table 2 show that symptoms of shock were invariably manifested with the larger doses of horse serum (2.0, 3.0 and 4.0 cc.) and never with the small doses (0.4 and 1.0 cc.). Between the extremes there is the 1.3 cc. dose which gave positive results in the primary and negative results in the duplicate series. Exactly as in series A, it is the middle zone which gives variable results. The variation was again attributed to individual differences in the diameter and distensibility of the intestines.

The loop length being uniform in this series, the extent of absorbing surface may also be taken as fairly uniform and, therefore, eliminated as a factor. This leaves increased intra-intestinal pressure as the factor of

importance. On purely mechanical grounds, this pressure should increase as the injected dose increases, provided the loop length, diameter, distensibility and amount of fluid secreted into the loop are fairly uniform in all trials. In the present series, as the dose is increased, that is, as the intra-intestinal pressure is raised, it passes from non-effectiveness to effectiveness in producing the symptoms of shock. It seems a clear and almost unavoidable inference that, given sufficient intra-intestinal pressure, the absorption and the sufficiently rapid absorption of undigested protein will take place.

Series C. Alternate variation of dose and length of loop. In the last series, 0.4 cc. was found to be a distinctly non-effective dose in a 15 cm. loop. In the present series, the effort was to see if such a dose could be made effective by shortening the loop, then to find a non-effective dose for this shortened loop and finally to make the second non-effective dose

TABLE 3

NUMBER	LENGTH OF LOOP	INTOXICATING DOSE	ANAPHYLACTIC SYMPTOMS	
			Primary series	Duplicate series
	cm.	*cc.*		
1	15	0.4	−	−
2	5	0.4	−	−
3	3	0.4	+	+
4	3	0.1	−	−
5	1	0.1	−	−
6	Intraperitoneal	0.4	+	+
7	Intraperitoneal	0.1	+	+
8	Intraperitoneal	0.01	−	−

effective by still further shortening of the loop. The procedure in other repects was the same as for the preceding series. The loop lengths, doses and results are given in table 3.

It is shown in this table that the decidedly non-effective dose of 0.4 cc. horse serum in a 15 cm. loop becomes effective in producing symptoms of shock when the length of loop is decreased to 3 cm. Furthermore, in such a 3 cm. loop the dose becomes non-effective when reduced to 0.1 cc. The only factor varying commensurately with such results is intra-intestinal pressure.

In guinea pig 5 an attempt was made to make the small dose of 0.1 cc. which was non-effective in the 3 cm. loop of guinea pig 4, effective by shortening the loop to 1 cm., care being taken to include an adequate blood supply in this short length. The result was, however, negative. No certain explanation can be offered without further investigation. Was the intra-intestinal pressure insufficient? The loop seemed distended to

a maximum on *post-mortem* examination; and it did not appear safe to try a shorter loop because the blood supply might be so interfered with as to prove inadequate for absorption. The guinea pig was properly sensitized as shown by a subsequent test. Was the 0.1 cc. dose subminimal for the particular degree of sensitization obtained by the animal in three weeks? A dose of 0.4 cc. and even of 0.1 cc. by intraperitoneal injection gave positive symptoms but not a 0.01 cc. dose, as shown by guinea pigs 6, 7 and 8. Was the dose then merely subminimal by the intestinal route? The results of later work reported below incline us to believe that not only is there a subminimal dose for the particular degree of sensitization of the animal but that this dose is higher by the intestinal route than by any

TABLE 4

NUMBER	LENGTH OF LOOP	INTOXICATING DOSE	DISTRIBUTION	ANAPHYLACTIC SYMPTOMS	
				Primary series	Duplicate series
	cm.	*cc.*	*cc./cm.*		
1	Unligated	1.3	0.00+	−	−
2	15	0.4	0.027	−	−
3	3	0.1	0.033	−	−
4	20	1.3	0.065	−	−
5	15	1.0	0.067	−	−
6	5	0.4	0.080	−	−
7	15	1.3	0.087	−	+
8	1	0.1	0.100	−	−
9	12	1.3	0.108	+	−
10	10	1.3	0.130	+	+
11	3	0.4	0.133	+	+
12	15	2.0	0.133	+	+
13	7	1.3	0.170	+	+
14	15	3.0	0.200	+	+
15	5	1.3	0.260	+	+
16	15	4.0	0.267	+	+

other. That is, the intestine offers more resistance to the absorption of the whole protein molecule.

Comparison of series A, B and C. It is interesting to arrange in tabular form all the results of the preceding series, using as a basis of comparison the number of cubic centimeters of horse serum per centimeter of loop length in each case. Table 4 shows such a comparison together with the positive or negative symptoms manifested in each case for both the primary and duplicate series.

By such a comparison it is clear that the results of the three series, both primary and duplicate, are in essential agreement. Invariably negative symptoms are displayed with small doses per centimeter and invariably positive symptoms with large doses. Given intestines of fairly uniform

diameter and distensibility this must mean that the intra-intestinal pressure per centimeter of length is less with the small doses and greater with the larger ones and that this is, therefore, the important factor in the absorption of the protein. As in the individual series, so in this combined comparative series the midzone of dosage per centimeter shows some difference between the primary and duplicate series and the duplicate series presents some irregularities in that the transition from negative to positive symptoms is not sharply defined. This should not be surprising. Individual differences between guinea pigs are to be expected; and furthermore, our methods though sufficiently delicate do not bear the mathematical treatment possible with, let us say, quantitative chemical analysis.

Series D. Constant length of loop with constant dose of horse serum increased to different total volumes by the addition of water. This series was undertaken to see whether a certain length of loop and dose of horse serum which in series A gave negative results, namely, 15 cm. of loop and 1.3 cc. of horse serum, could be made invariably or reliably effective by adding

TABLE 5

NUMBER	LENGTH OF LOOP	INTOXICATING DOSE	ANAPHYLACTIC SYMPTOMS	
			Primary series	Duplicate series
	cm.	*cc.*		
1	15	1.3	−	−
2	15	1.3 + 0.7 H_2O	−	+
3	15	1.3 + 1.7 H_2O	+	+
4	15	1.3 + 2.7 H_2O	+	+

water to the volume of horse serum, thus increasing merely the intra-testinal pressure.

Four guinea pigs comprised the series which was, however, performed in duplicate. The amount of water added to the 1.3 cc. horse serum varied between 0.4 and 2.7 cc. making up a total volume of 1.7 to 4.0 cc. In all other respects the procedure was the same as for series B. The results are presented in table 5.

Evidently the dose of 1.3 cc. is sufficient in itself in a 15 cm. loop to be reliably effective if only the pressure under which it is presented to the absorbing surface of the intestine is high enough. This was found to be the case when the total volume injected amounted at least to 3.0 cc. In series B, however, the minimal reliably effective dose was found to be 2.0 cc. Absolute quantitative agreement between the two series is not to be expected. Indeed, one might suspect that a little less pressure is required when the dose injected consists of pure horse serum than when it is diluted with water.

Series E. Measuring the intra-intestinal pressure. It will be recalled that the variable result in the middle of a series was attributed largely to individual differences in the diameter and the distensibility of the intestines. It seemed worth while to investigate these differences by directly determining, if possible, the intra-intestinal pressure developed with a given dose. Before we could begin this work an interval of about two months elapsed and we still had on hand a considerable number of guinea pigs which by this time were sensitized through a period of about 84 days instead of the usual 21 days. We determined to use them for this proposed work only to discover later that they no longer could serve our first purpose. Nevertheless this departure from our avowed method led to some interesting findings which are here reported.

The procedure in the series differed from previous ones in the following details. Having ligated both ends of a 15 cm. loop of fairly empty small

TABLE 6

NUMBER	LENGTH OF LOOP	INTRA-INTESTINAL PRESSURE	INTOXICATING DOSE	ANAPHYLACTIC SYMPTOMS
	cm.	*mm.*	*cc.*	
1	15	40	0.85	+
2	15	26	2.10	+
3	15	17	0.50	+
4	15	0	1.30	+
5	15	0	1.00	+
6	15	0	0.90	+
7	15	0	0.80	+
8	15	0	0.60	+
9	15	minus	0.50	+
10	15	minus	0.30	+

intestine, a burette (5 mm. internal diameter and graduated in tenths of a cubic centimeter and also in millimeters of height) was tied into the loop at one extreme end. At the other end was injected an amount of horse serum which filled the loop and rose to a certain height in the burette. The loop was then replaced in the abdomen and the cut edges of the belly wall approximated and temporarily held together by a clamp. The serum in the burette thereby rose to a greater height. By deducting the volume that rose in the burette from the volume injected, it was possible to know fairly accurately the amount of serum left in the loop. By reading the millimeters of height to which the serum rose in the burette, the pressure of the serum in the loop could be assigned a numerical value. Having taken these readings, the burette was tied off the loop and removed. The abdominal incision was then sutured and the animal allowed to come out of the anesthetic.

This procedure was repeated on four guinea pigs, varying the measurable

pressure from 40 mm. to zero. It was expected to find a pressure below which no positive symptoms of shock would obtain.

As appears in the results, table 6, this expectation was not fulfilled. Positive symptoms were manifested in every case. To give further assurance of this fact and also to determine individual differences in the volume capacity of the loops of small intestine, four more trials were made, injecting only enough serum so as just not to appear in the burette (zero measurable pressure) and two more trials at a minus pressure, that is, injecting less than the amount necessary for zero pressure. The results are also included in table 6. They were, to our surprise, likewise consistently or rather persistently positive. If the doses of serum are examined it will be noted that at least six were less than 1 cc. Now in series B, in which 15 cm. loops were also used, no dose less than 2.0 cc. gave positive results. It was realized, therefore, that these guinea pigs were not comparable with those of previous series. The only difference of which we were aware lay in the fact that the latter had been sensitized for a period of about 84 days instead of the usual 21 days. The significance of this difference in the period of sensitization will be brought out in the next series.

In regard to individual differences in small intestines, the results of our zero pressure measurements are instructive (trial 4 to 8 inclusive, in table 6). Under the given conditions the amount of serum required to just fill the loop, that is, without distending the walls, varied within the quite appreciable limits of 0.6 cc. and 1.3 cc. No cases above or below these limits were observed. It follows that one loop may hold as much as 0.7 cc. more than another of the same length (15 cm.) at zero pressure. This fact in itself is sufficient to account for our middle zone of variable results in the first three series.

Series F. Degree of sensitivity. The fact that the guinea pigs of the last series which had been sensitized for a period of about 84 days gave only positive results even with very small doses, suggested that they were nore highly sensitized. It seemed desirable to test the degree of sensitivity of these particular animals. This was done by injecting in a set of guinea pigs smaller and smaller amounts of horse serum into progressively longer loops of intestine, disregarding the pressure factor for the time. Finally the same decreasing doses were injected intraperitoneally into another set of guinea pigs and the results compared with those obtained by intestinal injection. Other points of procedure were the same as for series A and B.

Two facts of considerable importance emerge from the results of this series as summarized in table 7. First, these guinea pigs were apparently so highly sensitized that on intraperitoneal injection they responded with slight but distinctly positive symptoms of shock to a dose of horse serum as small as the original sensitizing dose, namely, 0.01 cc. Secondly, they reacted positively to as little as 0.40 cc. horse serum injected into the

unligated intestine; whereas our experience with guinea pigs sensitized for a short period had shown that from 5.0 to 15.0 cc. in the unligated intestine were required to get positive symptoms. This fact of extraordinary sensitivity of the animals accounts for the persistently positive results in the foregoing series E. It does not, however, discredit our factor of intra-intestinal pressure because we believe that the importance of this factor was amply demonstrated for the conditions holding in series A, B, C and D. It should be understood merely that whenever for some reason, for example, stasis, there is an unusual increase of intra-intestinal pressure, a sufficiently large amount of undigested protein will be absorbed and will be absorbed rapidly enough to result in overt symptoms of shock in guinea pigs sensitized for a period of about 21 days.

TABLE 7

NUMBER	INTOXICATING DOSE	INTRA-INTESTINAL ROUTE		INTRAPERITONEAL ROUTE
		Length of loop	Anaphylactic symptoms	Anaphylactic symptoms
	cc.	*cm.*		
1	1.3	15	+	+
2	0.6	15	+	+
3	0.3	15	+	+
4	1.3	30	+	+
5	1.3	60	+	+
6	1.3	Unligated	+	+
7	0.4	Unligated	+	+
8	0.1	Unligated	−	+
9	0.01	Unligated	−	+

What then is the significance of the fact that in the present series of highly sensitized animals as little as 0.40 cc. horse serum in the unligated intestine produces symptoms of shock? Can there be question here of unusually increased pressure? The unligated intestines of guinea pigs, nos. 6 and 7, were examined *post mortem* and found to be filled but not highly distended for considerable lengths with a clear fluid secreted into them. The effect of the weight of the intestine, breathing and peristalsis on parts of the gut filled with fluid may have been the establishing of some intra-intestinal pressure although, no doubt, much less than on a ligated loop and hardly to be considered extraordinary. The experiments on the highly sensitized animals would differ from those on less sensitized ones only in that a smaller amount of whole protein had to be absorbed in order to yield positive symptoms. Conversely, in the experiments on the less sensitized animals a greater amount was necessary or the absorption had to take place more rapidly—conditions brought about only by extraordinary increase in intra-intestinal pressure.

A further point already mentioned in series C is again brought out in the present series, namely, that the minimal effective dose via the intestinal route is higher (0.40 cc. in the unligated gut) than the minimal effective dose via the peritoneal cavity (about 0.01 cc.). The inference may be that the intestinal wall offers greater resistance to the absorption of whole protein than does the peritoneal wall—a statement simple and apparently quite in accord with accepted opinion. What we would emphasize, however, is that the resistance offered by the intestinal wall in comparison with other absorbing surfaces of the body is after all only a question of degree. Reviewing the fact that in our series of highly sensitized animals the whole protein molecule seems to be absorbed from the unligated intestine under conditions in which the intra-intestinal pressure can only be surmised and surely not considered extraordinary or abnormal, is not the possibility open to us that whole protein in minute amounts is regularly absorbed from the intestine? Our biological test of direct anaphylaxis may be far more delicate in detecting such minute amounts than any chemical test. We are somewhat loathe to proclaim this view without further investigation but no other seems quite equally capable of explaining our present observations and those on sensitization via the intestine, reported in the previous paper.

If our view based on these experiments has a general application to anaphylactic reactions in animals and human beings supposedly induced by absorption from the intestinal tract our explanation would be the following. Minute amounts of whole protein being regularly absorbed from the intestine, a condition of immunity will be rapidly instituted in the various tissues of the body for all proteins regularly or frequently ingested. When, however, a long interval, perhaps months, elapses between the very first ingestion of a particular protein and the next, a condition of sensitivity is set up so that the next ingestion may have a toxic effect depending on *a*, the length of the period of sensitization, *b*, the amount ingested, *c*, the influence of some condition causing stasis with increased intra-intestinal pressure and *d*, injury to the intestinal wall. It should be possible to obtain further evidence for this view by suitably planned experiments in which intoxication is tested after carefully controlled periods of sensitization, the attempt being made to accomplish both sensitization and intoxication by administering the protein by stomach tube. Work along these lines is now in progress and will be reported later.

Against our view that the whole protein molecule is absorbed into the circulation may be urged the possibility that the protein is first broken down intra-cellularly in its passage through the wall of the intestine and then resynthesized into something sufficiently akin to the original protein to yield the symptoms of anaphylactic shock. Although it is known that considerable protein digestion may and does take place during the passage

through the intestinal wall there appears to be no satisfactory evidence that a resynthesis is accomplished before absorption into the blood stream nor that the later synthesis results in a product even remotely resembling the ingested protein. Studies on anaphylaxis have shown, moreover, that the reaction is quite specific for a particular protein so that any considerable modification of the protein used for sensitization will destroy its intoxicating capacity. Finally, the fact that intra-intestinal pressure is an important factor in obtaining a sufficient or sufficiently rapid absorption for the display of shock in guinea pigs of low sensitivity would point to an inter- rather than an intra-cellular passage under our given conditions. Even though it is granted that in normal digestion most protein is absorbed by the intra-cellular route, there is no reason to suppose that minute amounts cannot even normally escape into the blood stream by an inter-cellular passage. It remains, therefore, our opinion for the time on the basis of our experiments that minute amounts of whole protein are regularly absorbed presumably by an inter-cellular route and that this absorption is appreciably increased by an increase of the intra-intestinal pressure.

SUMMARY

The observations reported were made on guinea pigs of *A*, moderate sensitivity, and *B*, high sensitivity to the foreign protein horse serum.

A. Guinea pigs of moderate sensitivity, that is, intraperitoneally sensitized with 0.01 cc. horse serum 21 days previously.

1. The injection of 1.3 cc. horse serum into the small intestine was found to be non-effective in causing symptoms of anaphylactic shock.

2. By injecting this non-effective dose into progressively shorter *ligated* loops of small intestine (from 20 to 5 cm.) in a series of guinea pigs, it was found to become effective in causing symptoms of shock when the loop length was 10 cm. or shorter.

3. Conversely, by using a constant length of ligated loop (15 cm.) and injecting progressively larger doses of horse serum (from 0.4 to 4.0 cc.) these were observed to change from non-effectiveness to effectiveness in the production of shock when the dose was 2.0 cc. or more.

4. A non-effective dose for a certain length of ligated loop (0.4 cc. for 15 cm.) was made effective by shortening the loop to 3 cm. By decreasing the dose for this shortened length of loop it was made non-effective again.

5. A non-effective dose for a certain length of ligated loop (1.3 cc. for 15 cm.) was made effective by the addition of sufficient water (to give a total fluid dose of at least 3.0 cc.).

The only factor consistently adequate to account for the results in the above series of observations is unusually increased intra-intestinal pressure which is, therefore, held to be the important factor for the sufficient and sufficiently rapid absorption of horse serum from the intestine to cause symptoms of anaphylactic shock in guinea pigs of moderate sensitivity.

B. Guinea pigs of high sensitivity, that is, intraperitoneally sensitized to horse serum about 84 days previously.

1. Animals of this degree of sensitivity reacted positively on *intraperitoneal* injection to a dose of horse serum as small as the original sensitizing dose (0.01 cc.); whereas those of moderate sensitivity required at least 0.1 cc.

2. These animals reacted positively on injection of small doses of horse serum into extremely long ligated loops (60 cm.) and even to as little as 0.4 cc. into the *unligated* intestine, that is, under conditions of no extraordinary intra-intestinal pressure.

Consideration of all the above facts leads to the tentative conclusion that minute amounts of whole protein are easily, perhaps normally, absorbed from the intestinal tract presumably by an intercellular route and that such minute amounts are detectable by highly sensitized animals. Furthermore, when for some reason, for example stasis, the intra-intestinal pressure is raised this absorption of whole protein may be so increased as to be detectable even by moderately sensitized animals. Support is thereby given to the view that immunity to the proteins of food follows the frequent ingestion of them and anaphylactic sensitization and intoxication the relatively infrequent ingestion.

We wish to express our appreciation of the constructive interest taken in this work by Dr. P. F. Swindle and Dr. H. Beckman.

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STUDIES IN REACTION TIME

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Reaction time has been investigated from almost every angle and the results of these studies are so well known that a present review of them is unnecessary.

In carrying out the investigation reported in this paper the writers have attempted to show the effect of systematic exercise on reaction time. Since the change in reaction time seemed to run parallel to a change of tonus in skeletal muscle, it seemed that by comparing tonic changes with variations in reaction time, something might be added to the interpretation of reaction time variations.

Auditory reaction time was recorded by the use of a Bergström chronoscope (Bergström, 1900). This type of chronoscope consists of a pendulum to which is attached a hand which points to a scale divided into σ. The stimulus is given by the experimenter by pressing a key which at the same time magnetically releases the pendulum. The subject is instructed to press a second key as soon as the stimulus is heard. This key being in series with a magnet on the pendulum shaft magnetically stops the swinging hand. The reaction time is then read from the scale in σ. The impact of the armature with the magnets which release the pendulum is used as the stimulus.

The subjects were all trained in reaction time experiments by giving them sufficient practice so that their reaction time records were consistent. The general method of procedure was first to take a series of one hundred normal reactions. The subjects exercised systematically for definite periods, fifty reactions being recorded during the rest period.

The knee-jerk was used as an index to the tonus of the skeletal muscle. The apparatus used to record the knee-jerk and the method of calculating its extent has been described by one of the writers elsewhere (Tuttle, 1924). The knee-jerk records were taken during the rest periods either preceding or following the reaction time measurements.

The subjects were given uniform instructions in the reaction time experiments. After being comfortably seated at the chronoscope they were instructed as follows:

> When all is ready, you will be given the signal "get ready." Following this the auditory stimulus will be given. When you hear the stimulus, a click, press the key

as soon as you can and hold it down until signaled to release it by the words "all right."

The time between the signal and the stimulus was varied in order to eliminate the possibility of the subject being "set" for it. Before each series, each subject was given three trials.

Three types of exercise were used in the experimental series. Two subjects climbed stairs, six skipped the rope and two did full knee-bends.

The subjects who climbed the stairs performed the following routine of exercise in whole or in part:

1 trip down and up 54 steps followed by 7 minutes' rest
2 trips down and up 54 steps followed by 7 minutes' rest
3 trips down and up 54 steps followed by 7 minutes' rest
4 trips down and up 54 steps followed by 7 minutes' rest
5 trips down and up 54 steps followed by 7 minutes' rest
6 trips down and up 54 steps followed by 7 minutes' rest
7 trips down and up 54 steps followed by 7 minutes' rest

During the rest period the reaction time and knee-jerk records were made.

The subjects who skipped the rope performed the following routine of exercise in whole or in part:

¼ minute rope-skipping followed by 7 minutes' rest
½ minute rope-skipping followed by 7 minutes' rest
1 minute rope-skipping followed by 7 minutes' rest
2 minutes rope-skipping followed by 7 minutes' rest
4 minutes rope-skipping followed by 7 minutes' rest
6 minutes rope-skipping followed by 7 minutes' rest
8 minutes rope-skipping followed by 7 minutes' rest

As before, during the rest period, reaction time and knee-jerk records were made.

The subjects who did knee-bends carried out the following routine of work:

½ minute knee-bending followed by 7 minutes' rest
1 minute knee-bending followed by 7 minutes' rest
2 minutes knee-bending followed by 7 minutes' rest
4 minutes knee-bending followed by 7 minutes' rest

Reaction time and knee-jerk records were made during the rest periods.

The reaction time data obtained from the stair-climbing group are shown in table 1.

The data in table 1 show that for subject 2 there is first a gradual increase in the reaction time during periods 1 and 2, followed by a material decrease during period 3. The reaction time then increases with the onset of fatigue. In case of subject 3 the reaction time has a tendency toward increasing with the onset of fatigue.

The data obtained from the rope-skipping group are shown in table 2.

The data presented in table 2 show, in general, that following the periods of exercise there is a time when the reaction time becomes shorter than normal. In case of subject 9, this phenomenon makes its appearance during period 2; subject 11, during periods 2 and 3; subject 8, during period 1; subject 15, period 3; subject 10, periods 2 and 3; and subject 16, periods 2 and 3.

TABLE 1

Shows the comparative length of the reaction time after successive periods of stair-climbing

SUBJECT NUMBER	NORMAL	AVERAGE LENGTH OF REACTION TIME AFTER STAIR-CLIMBING						
		1	2	3	4	5	6	7
2	174.6	187.6	189.0	160.0	168.6	195.2		
3	146.3	148.4	152.6	157.8	147.6	159.0	156.2	156.6

TABLE 2

Shows the comparative length of the reaction time after successive periods of rope-skipping

SUBJECT NUMBER	NORMAL	AVERAGE LENGTH OF REACTION TIME AFTER ROPE-SKIPPING								
		1	2	3	4	5	6	7	8	9
9	165.0	175.0	127.4	148.6	160.2	193.8				
11	147.4	146.8	142.2	142.0	151.6	156.6	154.8	157.0	164.2	188.6
8	168.8	158.5	166.0	179.8	197.0	214.0	238.0			
15	158.1	160.4	164.0	155.8	186.8	167.0	181.2			
10	179.7	159.4	151.2	151.0	171.6	173.4	173.2	190.6		
16	167.7	165.6	145.0	146.6	193.2	163.6	157.6	163.8		

TABLE 3

Shows the comparative length of the reaction time after successive periods of knee-bending

SUBJECT NUMBER	NORMAL	AVERAGE LENGTH OF REACTION TIME AFTER KNEE-BENDING			
		1	2	3	4
18	176.0	173.4	166.2	182.4	236.8
19	159.7	164.0	137.2	141.4	190.8

The data obtained from the knee-bending group are shown in table 3.

The data in table 3 show a phenomenon similar to that pointed out in table 2. For subject 18 the reaction time is shortest during period 2, and for subject 19 the shortest reaction time was recorded during period 2.

In general, the data presented in tables 1, 2 and 3 seem to indicate that early in the exercise experiment there appears a period of reaction time

TABLE 4

Shows a comparison of the height of the knee-jerk with the length of the reaction time after successive periods of exercise

SUBJECT NUMBER		NORMAL	COMPARISON OF REACTION TIME AND KNEE-JERK AFTER EXERCISE									
			1	2	3	4	5	6	7	8	9	10
9	KJ	32	31	36	41	31	18					
	RT	165	175	127	148	160	194					
11	KJ	53	52	58	55	56	54	55	58	53	50	42
	RT	147	146	142	142	152	157	155	157	164	189	191
8	KJ	10	13	13	13	15	4	2				
	RT	169	159	166	180	197	214	238				
10	KJ	24	31	32	40	30	29	20	15			
	RT	180	159	151	151	172	173	173	191			
2	KJ	22	26	27	20	11	10					
	RT	175	188	189	160	169	195					
3	KJ	32	30	29	28	24	23	20	16			
	RT	146	148	153	158	148	159	156	157			

TABLE 5

Shows a comparison of the height of the knee-jerk and the length of the reaction time in the control series

SUBJECT NUMBER		NORMAL	COMPARISON OF REACTION TIME AND KNEE-JERK CONTROL SERIES				
			1	2	3	4	5
10	KJ	28.1	26.3	22.7	22.4	25.3	26.8
	RT	139.4	149.6	144.0	149.8	148.6	139.4
8	KJ	18.8	19.6	23.7	23.8	23.9	
	RT	155.7	156.8	154.6	164.2	153.8	
11	KJ	38.8	33.2	29.7	26.7	24.3	25.0
	RT	132.0	129.2	129.2	128.0	128.6	128.0
2	KJ	30.2	32.7	20.7	33.1	34.0	
	RT	154.2	166.8	172.8	150.6	154.6	
12	KJ	27.4	25.2	24.7	25.2	25.9	26.5
	RT	152.0	154.0	164.0	160.0	165.0	147.0

shorter than normal, followed by a gradual increase with the onset of fatigue. Since this same phenomenon is also evident in the knee-jerk

records, a comparison of the two sets of data is made in table 4, the entire group being presented together regardless of the type of exercise.

The data in table 4 show that for subject 2 the knee-jerk is highest during period 2, while the reaction time is slowest during period 3; for subject 3, the highest knee-jerk during the normal period, lowest reaction time during the normal period; subject 8, highest knee-jerk during period 4, lowest reaction time during 1; subject 9, highest knee-jerk during period 3, lowest reaction time during period 2; subject 10, highest knee-jerk during period 3, lowest reaction time during periods 2 and 3; subject 11, highest knee-jerk during period 2, lowest reaction time during periods 2 and 3.

The data further show that in two cases the knee-jerk reaches its maximum during the same period the reaction time reaches the minimum. In one case the knee-jerk reaches its maximum during the period preceding the one in which the reaction time reaches its minimum, while in one case just the reverse is true. In case of subject 8 the knee-jerk is highest during period 4 while the reaction time is shortest during period 1. Subject 3 shows from the beginning a gradual increase in reaction time and a gradual decrease in the height of the knee-jerk. In all cases, however, the shortest reaction time and the highest knee-jerk appear during the early periods. In all cases the reaction time was slowest when the knee-jerk was the lowest.

A comparison of the height of the knee-jerk and the length of the reaction time for subject 9 is shown in figure 5 and for subject 10 in figure 6.

These data are shown graphically in figures 1 and 2.

The figures show that for subject 9 the knee-jerk becomes highest during period 3 while the reaction time is shortest during period 2. The knee-

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Fig. 1. This is a graphical representation of the comparative effect of exercise on the extent of the knee-jerk and the length of the reaction time of subject 9. Scale abscissa = 1 mm. = ½ minute of exercise; ordinate, 1 mm. = 1 mm., extent of knee-jerk; for reaction time, 1 mm. = 1 sigma.

Fig. 2. This is a graphical representation of the comparative effect of exercise on the extent of the knee-jerk and the length of the reaction time of subject 10. Scale same as for figure 1.

Fig. 3. This is a graphical representation of the average extent of the knee-jerk compared with the average length of the reaction time of the control series. Scale same as for figure 1.

Fig. 4. This is a graphical representation of the average extent of the knee-jerk compared with the average length of the reaction time of the exercise series. Scale same as for figure 1.

Fig. 5. The knee-jerk record of subject 9 taken during periods of rope skipping. The numbers show the average reaction time in sigma for corresponding periods of exercise.

Fig. 6. The knee-jerk record of subject 10 taken during periods of rope skipping. The numbers show the average reaction time in sigma for corresponding periods of exercise.

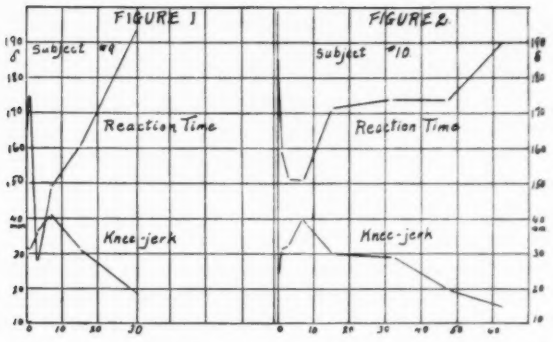
FIGURE 1
Subject #9
Reaction Time
Knee-jerk
FIGURE 2
Subject #10
Reaction Time
Knee-jerk

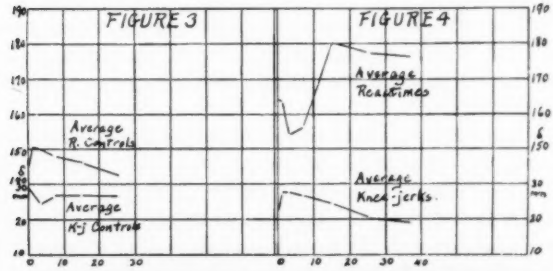
FIGURE 3
Average R. Controls
Average K-J Controls
FIGURE 4
Average Reactimes
Average Knee-jerks

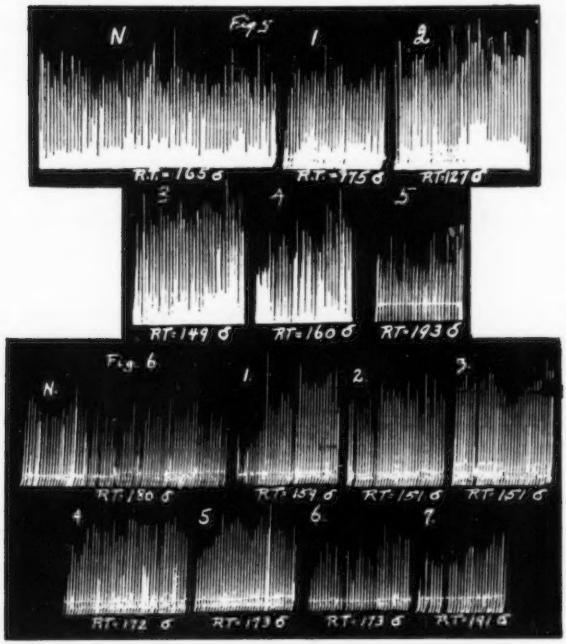
Fig. 5
N
1
2
R.T.=165 σ
R.T.=175 σ
RT:127 σ
3
4
5
RT:149 σ
RT=160 σ
RT:193 σ
Fig. 6
N.
1.
2.
3.
RT:150 σ
RT:159 σ
RT:151 σ
RT:151 σ
4
5
6.
7.
RT:172 σ
RT:173 σ
RT:173 σ
RT:191 σ

jerk was lowest during the same period the reaction time was longest, period 5. For subject 10 the knee-jerk was highest during the same period that the reaction time was shortest. The knee-jerk was lowest during the same period that the reaction time was longest, period 7.

In order to determine whether or not the characteristic features found in the reaction time and knee-jerk records were due to exercise a control experiment was carried out. Five subjects submitted to an experiment identical with the one previously described except that rest was substituted for exercise. The results of the control series are shown in table 5.

The data presented in table 5 do not show any of the characteristics of those presented in table 4.

Figure 3 shows graphically the average results of the control experiment, while figure 4 shows the comparison of the average height of the knee-jerk during exercise periods plotted against the average of the corresponding reaction time periods.

The data show that there is an initial rise in the reaction time control series while there is an initial fall in the knee-jerk control series (fig. 3). This is a characteristic usually present in knee-jerk and reaction time experiments. It is due to the fact that a little time is required for the subject to get adjusted to the experiment and to get away from the influence of other stimuli. In the exercise experiment the averages show that there is an initial fall in the reaction time series while there is an initial rise in the knee-jerk series.

CONCLUSIONS

1. During periods of controlled exercise, the reaction time first gets shorter and then longer, following the physiological sequence of "treppe" and fatigue.

2. During periods of controlled exercise, the knee-jerk first gets higher and then lower thus following the physiological sequence of "treppe" and fatigue (Brown and Tuttle, 1926).

3. The periods of decreased reaction time seem to correspond with the increase in the height of the knee-jerk; the period of increased reaction time seems to correspond with the period of decrease in the height of the knee-jerk.

4. Assuming that the knee-jerk is an index to the tonus of skeletal muscle, the data herein reported show that there is a direct relation between muscle tonus and reaction time.

5. The data presented above suggest that muscle tonus is either *a*, one of the factors controlling reaction time; *b*, a phenomenon, depending for its variation on the same factors upon which reaction time variations depend, or *c*, it is the factor controlling reaction time.

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ON THE INVERSE CHANGE BETWEEN THE CONCENTRATION OF GLUCOSE AND CHLORIDE IN THE BLOOD

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Histamine causes not only a fall of blood chlorides accompanying the secretion of HCl by the stomach as we reported recently (Lim and Ni, 1926), but also a rise in blood sugar. This led us to investigate as to whether the quantitative change of these two constituents was independent of each other, and whether a shift produced by factors other than histamine would show any reciprocal relationship. According to Herrick (1924), there is an inverse relationship between the concentration of glucose and chloride in the blood after the blood sugar has been raised by the ingestion of 100 grams of glucose. A perusal of data in which both glucose and chloride of the blood were estimated shows that instances of an inverse relationship are not uncommon.

Among the nephritic cases presented by Myers (1924) the blood of one patient (*P. L.*) exhibited such a relationship between glucose and chloride. Similar changes may also be observed in the blood of diabetic patients, according to the figures given by Gram (1923) and McLean (1915). Gram himself pointed out that in diabetics with high blood sugar the blood possesses a low conductivity due to a "secondary decrease of salts tending to keep down the high osmotic pressure caused by an increase of non-electrolytes" viz., sugar. In observations in 28 cases of diabetes mellitus, McLean (1915) found in the majority a lowering of the "chloride threshold." The observations mentioned above, however, were made on patients either after the ingestion of a fairly large quantity of glucose and water or under the condition of some serious pathological state.

In the experiments here recorded, attempts were made to cause movements of either the chloride or the sugar and to observe the effect on the other component of the supposed relationship.

METHODS. The experiments were carried out on dogs, under varying conditions, viz., normal, depancreatized, denervated adrenals, adrenalectomized, gastric pouches or fistulae, and intestinal fistulae. For the procedure employed on the animals with pouches, see Lim and Ni (1926).

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A fall of blood chlorides was experimentally produced either by partial obstruction of the intestine (Haden and Orr, 1923) or by stimulating the stomach (Lim and Ni, 1926). In the latter, two kinds of stimuli were used, psychic, by sham-feeding, and chemical, by the subcutaneous injection of (usually 0.2 mgm. per kilo) histamine acid phosphate. By these methods, the influence of meals and their contained chlorides and

TABLE 1

Single injections of histamine. Examples taken from Series I (N-2 and N-6), Series II (R-3 and R-4), Series III (P-20, P-26 and P-27)

DOG	TIME AFTER INJECTION	NaCl MGM. PER 100 CC. BLOOD	GLUCOSE MGM. PER 100 CC. BLOOD	DOG	TIME AFTER INJECTION	NaCl, MGM. PER 100 CC.			GLUCOSE, MGM. PER 100 CC.		
						Whole blood	Plasma	Corpuscles	Whole blood	Plasma	Corpuscles
	minutes				*minutes*						
N-2	Control	539	77	P-20	Control	561	692	256	109	110	106
	40	541	86		30	551	672	314	119	120	117
	105	506	84		120	557	685	256	118	118	116
					220	570	685	313	114	116	113
N-6	Control	564	71								
	20	537	74	P-26	Control	436	514	260	111	110	110
	40	241	77		34	425	509	239	112	113	113
	60	541	83		64	418	511	200	114	116	110
	80	528	81		84	475	526	361	110	110	109
	100	537	83		184	458	546	241	118	118	117
	120	551	91								
				P-27	Control	544	656	294	86	86	87
R-3	Control	577	131		35	518	617	334	91	98	77
	50	498	122		60	524	624	209	118	117	118
	110	511	116		115	508	627	142	115	119	106
					165	521	544	259	98	100	94
R-4	Control	564	91								
	40	528	100								
	100	531	104								

sugar were obviated. The fluctuations in the chloride level of the blood thus induced were supposed to be the primary factor. Changes in blood sugar as a primary factor were obtained either by the extirpation of the pancreas or by the injection of insulin.

The blood sugar was estimated and calculated as glucose by the Folin-Wu method (1920); the blood chlorides, by the Volhard method as modified by Whitehorn (1920). When data were also required on these two

constituents in the plasma as well as in the corpuscles, the blood was centrifuged immediately after collection, and the above methods carried out on the plasma, the corpuscle-value being calculated.

EXPERIMENTAL RESULTS: *Primary movement of chloride. Single injections of histamine (HCl secretion)*: Out of six normal dogs which were made to secrete gastric juice, by histamine injections, a rise in blood sugar was found in four animals. Three out of these four dogs showed a fall in blood chlorides. The inverse relationship was not marked in any (table 1). It may be mentioned that the gastric juice was not collected in this series, i.e., the animals were not subjected to the irritation of having a stomach tube passed by mouth.

An increase in blood sugar was observed in six out of the seven Pavlov-pouch dog experiments. As these animals were well accustomed to the technique employed, the opportunity for "emotional" disturbance was minimal. In the above six animals which showed a rise in blood sugar, a fall in blood chlorides was found in five and a rise in one. The decrease in chlorides occurred in both plasma and corpuscle (one exception), and a comparatively greater drop was often observed in the latter. The rise in sugar also took place both in plasma and corpuscle; but the increase was relatively greater either in the plasma (two cases) or in the corpuscle (also two cases).

Three of the four normal dogs, in which the entire gastric juice was continuously aspirated through a Rehfuss tube, showed a rise in blood sugar; a fall in blood chloride was observed in all these four animals after the histamine injection. While the increase in sugar was not great, a large fluctuation in the chloride of the whole blood occurred in two of these cases.

Although the results seem to show an inverse relationship between the concentration of glucose and chlorides in the blood of some dogs following "histamine" (i.e., primary fall of chloride) such reciprocal relationship was neither constant nor in any proper proportion.

Repeated injections of histamine (prolonged HCl secretion): The glucose and the chloride in the blood of eight Pavlov-pouch dogs, which were being subjected to hourly injections (for 12 to 24 hours) of histamine (for other data see Lim and Liu, 1926) were examined at intervals of four or five hours. In the twelve-hour series (4 dogs), only one animal showed an increase in blood sugar.

A steady rise in the whole blood sugar, however, was invariably observed in the other four dogs subjected to hourly injections for twenty-four hours, as was a fall in chlorides (table 2).

Any attempt to balance this relationship on the basis of osmotic compensation must take into consideration the influence of the other constituents on the osmotic equilibrium. That the electrolyte molecules

and ions constitute nearly all of the osmotically active substances present in the blood is shown by the correspondence between the lowering of vapor tension observed (Neuhausen, 1922) and the lowering attributable to the electrolytes. Glucose is present in such small amounts in normal blood, that it does not play an important rôle in controlling the osmotic pressure. It is still of interest, however, to determine how far under our experimental conditions the compensation hypothesis is tenable.

TABLE 2

Hourly (P-20, P-22 and P-23) and half-hourly† injections (G-17 and L-1) of histamine*

DOG	TIME AFTER 1ST INJECTION	NaCl, MGM. PER 100 CC. OF WHOLE BLOOD	GLUCOSE, MGM. PER 100 CC. OF WHOLE BLOOD	DOG	TIME AFTER 1ST INJECTION	NaCl, MGM. PER 100 CC.			GLUCOSE, MGM. PER 100 CC.		
						Whole blood	Plasma	Corpuscle	Whole blood	Plasma	Corpuscle
	hours				*minutes*						
P-20	Control	567	96	G-17‡	Control	508	594	594	93	92	93
	5	561	98		60	511	605	388	100	125	70
	10	495	112		180	429	511	333	91	118	55
	15	475	127		300	398	514	238	100	133	61
	20	478	143		420	359	452	261	101	153	44
					570	316	402	221	80	87	74
P-22	Control	501	86								
	5	468	95	L-1	Control	467	574	346	99	99	100
	10	462	111		60	475	561	434	96	141	51
	15	458	111		130	422	528	316	95	125	60
	20	449	115		150	452	552	353	83	100	66
P-23	Control	498	100								
	5	488	109								
	10	468	102								
	15	372	126								
	20	376	133								

* 0.2 mgm. per kilo per hour.

† 0.5–1.8 mgm. per kilo per half-hour.

‡ This animal has both adrenals denervated.

Thus, in dog *P. 25*, there was a typical inverse change between the concentration of the glucose and the chlorides. The increase in milligrams of glucose per 100 cc. of whole blood (taken at intervals of five hours) above the control value was 33, 57, 57 and 55. The decrease in chloride of the corresponding samples was 23, 79, 112, 106 mgm. (in terms of NaCl) per 100 cc. If we assume that, with the exception of glucose and chlorides, everything else remained unchanged, and that the chloride lost

was NaCl,[2] then the osmotic changes produced by the fall of chloride would be equivalent to 0.175, 0.603, 0.844, and 0.809 atmospheres[3] (corrected for the isotonic coefficient[4]) while the changes caused by the rise

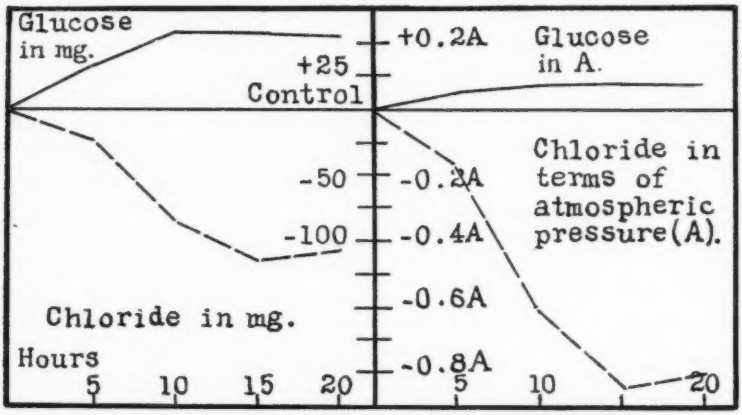

Fig. 1. Example from dog P 25 showing the inadequacy of compensation between glucose and chloride.

of glucose in the corresponding samples, to 0.047, 0.081, 0.081 and 0.078 A[5] respectively (fig. 1).

[2] Since NaCl and KCl are dissociated to nearly the same extent, the last supposition would not cause a great error.

[3] Hereafter designated as A.

[4] The isotonic coefficients are referred to that of sucrose taken as unity (quoted from Physical Chemistry, 1921, 2nd edition, p. 72). This is only approximate, since there are "residual reducing substances" (about 0.01 to 0.03 per cent) which are not glucose (Hiller, Linder and Van Slyke, 1925), and since the degree of dissociation of NaCl in the blood is assumed to be the same as in water, (Neuhausen and Marshall (1922) have estimated that the Na salts in blood serum are 83 per cent dissociated, so that our assumption is close to the fact).

[5] As was demonstrated by Van't Hoff, the Boyle-Gay-Lussac law for gases and the Avogadro hypothesis could be extended to solutions. The osmotic pressure of glucose and of chloride, therefore, may be calculated by the formula:

$$P = \frac{W \times 2.02 \times 1000 \times 22.4 \times (273 + t)}{M \times 100 \times 2.02 \times 273}$$

Where P is the osmotic pressure, W the weight (grams of glucose or of NaCl in 100 cc. of blood t the body temperature, and M the molecular weight of glucose (or of NaCl). Morse and his co-workers have shown that the osmotic pressure of glucose agrees within 6 per cent with the values demanded by the Van't Hoff theory, being 6 per cent larger for concentrations ranging from 0.1 to 1.0 molar. Since the glucose concentration in the blood of non-diabetic dogs is usually lower than 0.01 molar, the calculated osmotic pressure as it is presented here, would be fairly close to the actual value.

In another example, dog *L 1*, (the entire stomach was formed into a pouch, so that the entire HCl secretion was lost; this animal received half-hourly injections of histamine) both the whole blood and the plasma were analyzed, the value for the corpuscles being calculated. The blood volume was determined from the hemoglobin and hematocrit readings. Since the plasma value of the two constituents, being directly estimated, was more accurate and since the greatest rise of glucose occurred in it, we may examine the inverse change which have taken place in the plasma. The estimated increase in plasma glucose (table 2) was 42 mgm. per 100 cc. plasma at the end of one hour. The plasma volume was 304 cc. before injection and 261 cc. at the end of the first hour. The actual increase in plasma glucose was therefore 25 mgm. per 100 cc. of plasma, allowance being made for the change of the blood volume and that of the relative

TABLE 3

Sham-feeding (S-3 and S-6) and partial intestinal obstruction (O-1)

DOG	TIME	NaCl, MGM. PER 100 CC. BLOOD	GLUCOSE, MGM. PER 100 CC. BLOOD	DOG	TIME	NaCl, MGM. PER 100 CC. BLOOD*	GLUCOSE, MGM. PER 100 CC. BLOOD*	DOG	TIME	NaCl, MGM. PER 100 CC. BLOOD	GLUCOSE, MGM. PER 100 CC. BLOOD
S-3	9:50	531	141	S-6	9:33	478	186	O-1	9:40	590	88
	10:15	Sham-feeding			9:40	Sham-feeding			10:10	Intestinal obstruction	
	10:55	495	166		10:00	452	220		4:10	580	97
	11:25	Sham-feeding			10:30	454	227		2nd day	508	105
	11:55	490	162		11:00	454	215		3rd day	597	87
	12:25	508	143		11:30	454	202		4th day	627	83
	1:25	498	143		12:00	452	190		6th day	541	74
	2:25	518	143		12:30	481	180				

* All blood samples were taken from the left heart.

volume between plasma and corpuscle. The decrease in the chlorides at the corresponding time was 107 mgm. per 100 cc. of plasma. If we assume that during the first hour, the other substances maintain constant relations to each other, then the osmotic disturbance produced by the fall of the plasma NaCl is equivalent to 0.816 A, and the osmotic change caused by the rise of glucose, equivalent to 0.035 A. The latter is thus too small to compensate for the former. Our experimental data, therefore, show that the seemingly inverse relation between the concentration of glucose and chloride, after the injection of histamine, cannot be attributed to an osmotic compensation, and *that the rise of sugar is probably independent of the primary fall of chloride in the blood.* The following experiments tend to support this conclusion.

Sham-feeding: These results show that the movement of sugar and chloride is not due to histamine *per se* (table 3); here the inverse change is also present.

Partial obstruction of the intestine: A partial obstruction of the duodenum lowered the blood chlorides. Later, following the isolation and closing of a loop of duodenum in the same animal a further drop occurred. The inverse change of blood sugar was not always observed. In another

TABLE 4

Before and after the denervation of both adrenal glands (A-2 and A-4), and after the removal of both adrenals (A-5)

	BEFORE DENERVATION			AFTER DENERVATION			AFTER ADRENALECTOMY			
DOG	TIME	NaCl, mgm. per 100 cc.	Glucose, mgm. per 100 cc.	TIME	NaCl, mgm. per 100 cc.	Glucose, mgm. per 100 cc.	DOG	TIME	NaCl, mgm. per 100 cc.	Glucose, mgm. per 100 cc.
A-2	2:58	495	98	10:55	511	91	A-5	Feb. 23 7:15	Adrenalectomy	
	3:03	Histamine		11:00	Histamine			Feb. 24 11:10	498	88
	4:10	471	103	11:50	*504	*95		11:15	Histamine	
	4:33	462	104	12:35	*521	*94		11:45	511	88
A-4	11:30	514	84	12:25	541	91		12:15	504	85
	11:35	Histamine		12:35	Histamine			1:00	491	86
	12:30	488	88	1:35	498	90		Feb. 25† 11:20	554	82
	12:55	491	96	2:00	531	93		11:32	Histamine	
A-4	10:37	501	96	10:50	511	91		12:00	551	81
	10:40	Histamine		10:55	Histamine			1:00	495	77
	11:30	488	101	11:33	498	88				
	12:10	462	102	12:00	511	91				

* Blood samples were taken from the left heart.

† Gastric secretion collected.

dog, a partial obstruction of the ileum caused, especially on the second day, a marked fall of blood chlorides. The changes in sugar level were comparatively slight (table 3).

Denervation of both adrenal glands: Whether a change of the chloride level in the blood could take place independently of the rise of blood sugar, or whether the latter could be prevented, without affecting the former, was ascertained in dogs with "denervated" adrenals. The usual amount

of histamine (0.2 mgm. per kilo) did not cause the same increase of blood sugar as before, although the lowering of blood chloride was as evident as normal (table 4). This difference may possibly be regarded to be within the limit of experimental error, but the result of the succeeding experiment is unquestionable.

Double adrenalectomy: Histamine was injected into an adrenalectomized dog about sixteen and forty hours after the adrenals had been removed (the animal survived for 73 hours). In both observations the rise of blood sugar was prevented by the double adrenalectomy, while the fall of the blood chloride was still taking place (in one case the fall being as great as 59 mgm. (NaCl) per 100 cc. of whole blood) (table 4). The fact that, after the injection of histamine, there was a great fall of blood chloride accompanying a marked increase in HCl secretion (from 45 mgm. per hour to 147 mgm. per hour, an increase of 222 per cent) proved that the dog did respond very well to histamine.

These last experiments, therefore, appear to support the view that, while histamine (HCl secretion) causes the decrease in the blood chloride, the simultaneous rise in the blood sugar is independent of the chloride fall but dependent, at least in great part, on the integrity of the adrenal-sympathetic apparatus.

Primary movement of glucose. Removal of the pancreas: Large fluctuations of glucose level can be induced either by pancreatectomy or by insulin injections (see below) without introducing either sugar or water into the circulation of the animal.

After total extirpation of the pancreas, there was a great increase in blood sugar accompanied by a decrease in the blood chloride. A subcutaneous injection of 20 units of insulin lowered the sugar from 332 mgm. to 182 mgm. per 100 cc. of whole blood, at the end of one hour, and 49 mgm. at the end of five hours and ten minutes; the corresponding chloride value rose from 521 mgm. to 554 mgm. and then to 594 mgm. respectively (table 5). The inverse change shown here was remarkable. The high glucose level after the extirpation was 254 mgm. and 332 mgm. per cent, being an increase of 78 mgm. per 100 cc., which would be osmotically equivalent to 0.111 A. Assuming that other conditions remain constant, the osmotic change caused by the fall of the chloride of the corresponding blood sample was 0.053 A. Here, the influence of the rise of sugar on the osmotic balance is not offset by the decrease of chloride, so that if the movement of the latter is an attempt at compensation, it is about 50 per cent inadequate. It may be noted in passing that in diabetes with high blood sugar, the osmotic pressure is also raised (Gram, 1923), showing that chloride movement, which may have occurred, does not fully compensate for the increase due to sugar.

Insulin: Inverse changes between glucose and chloride, after the in-

jection of insulin were also observed in six non-diabetic dogs. In the diabetic dog, an injection of insulin brought the blood sugar down from 332 mgm. to 182 mgm. per 100 cc. of whole blood, the sugar reduction having an osmotic equivalent of 0.213 A. The accompanying rise of chloride was equivalent to 0.252 A. This is in fact an over-compensation, and is in contrast with the results obtained after the injection of histamine. In nearly all the observations made on the six non-diabetic dogs, the fall of glucose in the whole blood after insulin is over-compensated by the rise of the chloride level, if the data are calculated in terms of atmospheric

TABLE 5

Removal of pancreas (D-1) and injection of insulin (I-3 and I-6)

DOG	TIME	NaCl, MGM. PER 100 CC. BLOOD	GLUCOSE, MGM. PER 100 CC. BLOOD	DOG	TIME	NaCl, MGM. PER 100 CC.			GLUCOSE, MGM. PER 100 CC.		
						Whole blood	Plasma	Corpuscle	Whole blood	Plasma	Corpuscle
D-1	Feb. 22 10:12	Pancreatectomy		I-3	9:15	574	705	405	91	91	90
	6:00	528	254		9:20	Insulin 20 units					
	Feb. 23 10:40	521	332		10:40	600	727	410	50	48	52
	10:50	Insulin 20 units			1:30	587	698	420	58	48	72
	11:50	554	182		12:50	580	685	480	52	53	50
	4:00	594	49	I-6	9:58	551	660	388	97	96	98
					10:00	Insulin 20 units					
					10:30	577			83		
					11:30	567	643	462	59	52	69
					1:58	547	630	433	71	66	84

pressure. This does not hold good for the inverse changes occurring in the plasma (table 5).[6] The results of the above experiments suggest that the inverse changes between these two blood constituents are related to each other. It may be concluded, therefore, that a primary movement of glucose will influence the movement of chlorides in the opposite direction, without implying that a primary movement of chloride has a similar effect on glucose.

[6] In spite of the fact that the rise in chloride is assumed to be a compensating movement, and that its extent is adequate enough to justify such an assumption, the influence of other factors, e.g., CO_2, on the movement of Cl ions between corpuscle and plasma may play an not unimportant rôle. (Doisy, E. A., 1922; Meldanby, J., 1923).

SUMMARY

Histamine causes in dogs a fall of blood chloride (consequent on gastric secretion) which is frequently accompanied by a rise in sugar. This inverse change is more marked in the plasma than in the corpuscles.

Similar inverse changes may also be obtained by means of sham-feeding or by partial obstructions of the intestine, although the inverse relationship is not in invariable proportion.

Either denervation of the adrenal glands or double adrenalectomy prevents the rise in blood sugar by histamine, without materially affecting the fall in chloride.

After total extirpation of the pancreas, the subsequent hyperglycemia is accompanied by a marked lowering of the blood chlorides. An injection of insulin which brings the sugar down raises the blood chloride, in diabetic as well as in non-diabetic dogs.

Our results would suggest that while the increase in the blood sugar which accompanied a primary decrease in chlorides is probably the result of reflex stimulation of the adrenals, i.e., the sugar change is not dependent on the chloride, the fall and rise of blood chloride which occurs in pancreatectomized dogs, or normal dogs after the injection of insulin (primary movement of sugar) are directly related to the sugar fluctuations and may be due to an effort at osmotic and other compensations.

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THE RELATION OF PULSE PRESSURE TO STROKE VOLUME

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In 1904 Erlanger and Hooker suggested the use of the product of the pulse pressure × pulse rate as an index to the circulation rate. That the relation of these two values to one another, or more simply stated, of the pulse pressure to the stroke volume of the heart, might not be one of direct proportionality they fully realized. Direct proportionality, they pointed out, could only obtain provided the capacity and elasticity of the arterial reservoir, and the velocity with which the contracting heart empties itself into the arterial reservoir remain constant from beat to beat. Differences in the capacity of the arterial tree and in the elasticity of the arteries in different individuals, they realized, preclude the use of the pulse pressure × pulse rate for comparison of different circulations. The real question was in how far, in one and the same individual, variations in the size and the elasticity of the arteries and in the contraction time of the heart might alter the relation of pulse pressure to stroke volume from one of direct proportionality. In the absence of experimental data they regarded it best to employ the product of the pulse pressure × pulse rate merely as an index to the direction of changes in the circulation rate occurring from moment to moment and under conditions not far removed from the normal.

Since then a number of investigators have succeeded in securing experimental data bearing on this question. Dawson and Gorham (1908) concluded from direct measurements in anesthetized dogs that under normal conditions and during various procedures the pulse pressure was a reliable index of the systolic output. Henderson (1906) stated that "tracings support the proposition advanced by Erlanger that the pulse pressure is proportional to the systolic volume discharge of the heart." In a subsequent paper, however, Henderson (1908) retracted to the extent of admitting "a general proportionality in such tracings;" but a more careful analysis of the curves, he adds "shows very considerable deviation from exact proportionality." It should be noted that Erlanger and Hooker never maintained that there was, indeed their discussion of the problem made it perfectly clear that there could not be, an exact propor-

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tionality. Wiggers (1910) came to the same conclusion as did Erlanger and Hooker. He says that "The pulse pressure is never a quantitative measure of the systolic output; but, when compared to some immediately preceding observation, may generally be used as a qualitative indication of the direction in which the systolic discharge varies."

More recently Miss Skelton (1921) as a result of determinations carried out on the Starling heart-lung preparation, has come to the conclusion that the product pulse pressure × pulse rate (P.P. × P.R.) is of no value as a measure of the heart output. If it be true that, as Miss Skelton puts it, "Since a definite relation between the figures so obtained and the minute volume is very generally accepted at the present time by clinicians," it only can be added that clinicians have been assuming something that is unwarranted by the literature. Since Miss Skelton's conclusions differ so materially from those already on record an examination of her method and of her data seems desirable.

With regard to her method it would seem that the applicability of data derived from the heart-lung preparation to conditions obtaining in the intact organism is, to say the least, questionable. As has been said, the magnitude of the pulse pressure developed by the cardiac output depends among other things upon the capacity and the elasticity of the arterial reservoir. In Miss Skelton's experiments the magnitude of these two factors is determined by the volume and the pressure of air contained in a test tube; and data are lacking upon which an effort to estimate their significance could be based. Again in her experiments, the stroke volume determined by calculation from her data ranges between 1 and 13 cc. Now the literature points to 9 to 13 cc. as a probable normal resting stroke volume in dogs of the size she used. Most of her observations, therefore, are based upon outputs far below the lowest possible normal level, whereas the physiological range in normal animals probably is mainly above the resting level and does not exceed three to four times the resting stroke volume. Finally the range of arterial pressures obtaining in her experiments (systolic 256.8 to 73.5, diastolic 150 to 0) not only extends far beyond the normal, but in the case of the diastolic, at least, reaches a level that is incompatible with life.

Despite these serious objections to Miss Skelton's method as a means of testing the question at issue, in only four of her determinations does the output in consecutive determinations fail to change in the same direction as P.P. × P.R. The conditions obtaining in the case of these exceptions were as follows: *a*, Maximum-minimum blood pressure changed in the two successive determinations from 175.0–86.1 to 235.5–150.4 mm. Hg, that is, to extreme hypertension; *b*, diastolic pressures in two successive determinations 0.0 and 10.5 mm. Hg, pressures which are quite incompatible with life in the intact organism; *c*, maximum-minimum blood

pressures changed from 170.0–75.0 to 230.0–135.0, again to extreme hypertension; and *d*, maximum-minimum blood pressures changed from 230.0–135.0 to 73.5–5.0 mm. Hg, a fatal diastolic pressure. Clearly the change in the conditions in these four instances can scarcely be regarded as lying within the range of the normal and they do not, therefore, bear on the validity of the use of P.P. × P.R. as proposed by Erlanger and Hooker.

In view of the questions raised by Miss Skelton's work it seemed desirable to ascertain by additional experiments what the limitations are to the use of the product of pulse pressure × pulse rate as an index to volume flow. Inasmuch as we are now in the possession of practical methods for the determination in man of the systolic and diastolic blood pressures and of the circulation rate, which, when properly controlled and checked, are almost as accurate as those available for use in animal experimentation, it was decided to carry out the investigation on man as the subject.

Methods. *Blood pressure.* It was essential in these experiments to obtain dependable readings of the blood pressures. For this reason determinations were made simultaneously, and as frequently and as objectively as possible, by two methods, the Erlanger graphic and the auscultatory, the one serving as a check upon the other. The oscillations of the Erlanger instrument were recorded on a long roll kymographion together with the compressing pressure which was inscribed by a float riding on the mercury manometer. In addition the beginning of the first phase and the change from the third to the fourth phase of the Korotkoff sounds were electrically signalled on the same record. Subsequently the compressing pressures at which these two sets of systolic and diastolic signs had been registered were determined by mensuration of the records. The systolic signs, both oscillatory and auscultatory, as a rule were definite and in perfect agreement; but at times, in certain of the cases, difficulty was experienced in reading the diastolic pressure. At such times the auscultatory sign (dulling of the sounds) was indefinite; and then the oscillatory sign (inflection of the curve of decrease in amplitude of oscillations) also was difficult to recognize. Evidently this occasional indefiniteness of the two diastolic signs is due to one and the same cause, whatever it may be. The instances in which this difficulty were encountered are indicated in the table by the asterisks.

How large an error there is in the determination of the blood pressures it is impossible to say. Where the signs are clear they are unmistakable. In such cases the error in reading the pressure at which the signs register, probably is very small. If there is, in addition, a systematic error due to the value of the signs themselves it is impossible to estimate its magnitude. If it be assumed that it has the effect of making the pulse pressure either

too large or too small by 5 mm. Hg, the error in the case of the smaller pulse pressures (25 mm. Hg) would reach as a maximum ± 20 per cent; in the case of larger pulse pressures (45 to 50 mm. Hg or more) not over ± 10 per cent.

During each determination of the circulation rate ten readings of the arterial pressures were made except in the period following heavy exercise when only five were taken. The average of all the readings is taken as the blood pressure of the period.

Pulse rate. The pulse rate was obtained from the records made during the pressure determinations by counting the pulse waves recorded in a twenty second period.

Circulation rate. For the determination of the circulation rate the method of Henderson and Haggard (1925) was employed. The methods of Field, Bock, Gildea and Lathrop (1924) and of Burwell and Robinson (1924) were considered, but were not used because they involved a more complicated procedure from the standpoint of both the subject and operator, without apparently offering a greater degree of accuracy.

At the outset some difficulties were encountered with the Henderson and Haggard method, particularly in the conditioning of the iodine pentoxide tube. This process required in our experience between two and three weeks of daily heating of ten hours or more, instead of the total of twelve to twenty-four hours mentioned by Henderson and Haggard. We have conditioned five tubes, one of which was kindly sent us by Doctor Haggard, but the time required was practically the same for all, except in the case of the tubes we have prepared in a new way. Another difficulty consisted in the occasional liberation during the course of a determination of an unreasonable amount of iodine. A blank run immediately after such an occurrence also would be found rather high, indicating that the tube needed reconditioning. An explanation of this happening that occurred to us is that particles of iodine pentoxide become conditioned on the surface but may still enclose iodine which may be set free as with oxidation the particles undergo disintegration. In order to prepare a tube in which such occurrences would be minimized, we dissolved 30 grams of iodine pentoxide in 30 cc. of distilled water, and poured the solution into the U-tube containing the glass wool. The water was slowly evaporated by heating to about 65° in vacuo, before conditioning the tube at the higher temperature. At the end of fifteen hours of heating, the tube was completely conditioned. No abnormal values have been obtained with samples of air passed through such tubes. It is more convenient to make the tube smaller than the one originally described. The tube we used is 15 mm. in diameter; it is bent into a U 15 cm. high, and 8 cm. wide.

In our use of the method we have encountered two other difficulties. Our values for CO_2 in both the expired and the alveolar air were rather low.

By using an outlet tube to the room air from the expired-air mixing chamber 15 mm. in diameter and 30 cm. long, the values of CO_2 in the expired air were appreciably raised. More constant results and higher values for CO_2 in the alveolar air were obtained by reducing the air space above the fluid in the Müller valve to about 30 cc., by using a 1 per cent H_2SO_4 solution for the fluid, and by having a tube 10 mm. in diameter reach the surface of the acidulated water so that the capillary rise of fluid in this tube was less than 2 mm., thus reducing the resistance offered to the negative pressure produced by inspiration.

Subjects and order of procedure. Four normal subjects were studied, T. H. 17 years, W. D. 30 years, D. W. 21 years and E. E. 45 years. At least two complete sets of determinations were made on each subject, a set consisting of observations on four different conditions. Each condition was observed at least once in duplicate about an hour apart except in the case of exercise by one of the subjects (W. D.). The four conditions chosen were such as might exhibit definite but different circulation states. They were the recumbent and standing postures, and light and heavy exercise. In the first three conditions observations were carried out over a period of ten minutes, and in the last, five minutes. We would have preferred making observations during the active period of heavy exercise, but owing to the impossibility of obtaining blood pressure tracings the observations were made immediately afterward.

When observations were started on the subject in the recumbent state he had lain quietly for an hour or more. In the case of the standing state, observations were begun after the subject had stood quietly for a half-hour or more, except in the case of D. W., who stood more quietly than the others, but for not more than fifteen minutes without the supervention of faintness and vertigo. Light exercise was taken by the subject seated in the chair ergometer described by White (1924). The chair is bolted to a base and tilted slightly backward. The subject's head is supported by an adjustable headrest. The arm from which the arterial pressures were read, is supported by a shelf at the level of the heart. The legs are suspended in a nearly horizontal position. The exercise consisted in moving each leg back and forth about 50 times per minute, thus lifting a 7 kilo weight an average distance of 40 cm. Approximately 250 kilogram meters of mechanical work were done per minute. The observations were made during the active period of exercise after the subject had been exercising for ten minutes. For heavy exercise three of the subjects (D. W., W. D. and T. H.) ran for five minutes up and down a corridor 140 feet long, thus covering a distance of about $\frac{3}{5}$ of a mile. The fourth subject (E. E.), the oldest, stood, raised his arms over his head, bent forward, touched his toes, and straightened himself, repeating this movement rapidly for five minutes. It was not possible to provide a form of heavy exercise that would not interfere

with the determination of the blood pressures, but immediately after the exercise, the subjects lay on a table and observations were started as soon as possible and were continued for five minutes. The interval elapsing between the cessation of exercise and the beginning of the determinations averaged about fifty seconds but owing to certain difficulties the blood pressure readings were begun a little later than the determination of the circulation rate. A circulation rate of one period is, therefore, being compared in this set of observations with the pulse rate and pulse pressure of a slightly later period. Though the results obtained after exercise reveal the usual relationships, the information thus gained does not have the same value in relation to the problem in hand as that gathered in the other experiments in which the blood pressures and the circulation rate have been determined synchronously and during a steady state.

Results. Before considering the significant data in their relation to the problem in hand an attempt will be made to estimate the probable error of the method of determining the circulation rate. We have no way of attacking this question directly, but it is possible to approach it indirectly. There can be no doubt but that a given stroke volume will develop a constant pulse pressure, all of the pertinent conditions remaining constant. Therefore, in different determinations in one and the same subject the pulse pressure and the stroke volume should be essentially constant provided essentially similar diastolic blood pressures and pulse rates obtain, and provided also the condition of the subject has been such as to lead one to feel reasonably certain that the capacity and elasticity of the arterial tree have been constant. Any variation under such circumstances may in a large part be charged against experimental error in the determinations of the stroke volume and in the determination of the pulse pressure.

When the 58 determinations of the stroke volume listed in table 1 are examined with these considerations in mind two are found which are quite inconsistent with the remainder of the data, and can be accounted for only as being due to a gross error of some kind. This applies to observation T. H., 2/13, recumbent; here is found associated with usual blood pressures and heart rate a stroke volume of 54 cc., whereas the range of comparable stroke volumes is from 74 to 92 cc. And it applies also to determination W. D., 1/12, standing, which shows a stroke volume of 78 cc., whereas the four comparable determinations fall within the range of 50 to 65 cc. These data, indicated by the double asterisks in the table and underlined in the graph, are, therefore, excluded from further consideration and are not used in determining the averages. Certain other determinations, similarly marked, are not included in the averages of the data representative of some one of the four states studied, either because the subject was abnormal at the time or because the conditions were unusual. The experiments thus excluded are: 1, W. D., 12/15, because on that day

TABLE 1

SUBJECT	DATE	RECUMBENT							STANDING							LIGHT EXERCISE							POST-HEAVY EXERCISE						
		P.R.	D.P.	P.P.	S.V.	S.V./P.P.	P.P.×P.R.	C.R.	P.R.	D.P.	P.P.	S.V.	S.V./P.P.	P.P.×P.R.	C.R.	P.R.	D.P.	P.P.	S.V.	S.V./P.P.	P.P.×P.R.	C.R.	P.R.	D.P.	P.P.	S.V.	S.V./P.P.	P.P.×P.R.	C.R.
T.H.	12–16	(a) 70.4	77	41	80	1.95	2,888	5.6	(b) 88	83	27	49	1.82	2,375	4.3														
	12–21															110	79	47	90	1.91	5,180	9.9							
	1–14	(a) 77	71	45	82	1.82	3,465	6.3	(c) 110.4	89	25	33	1.32	2,760	3.6														
		(b) 76	72	45	79	1.75	3,420	5.7	(d) 104	87	27	35	1.30	2,810	3.6														
	1–15															(a) 108	80	45	69	1.53	4,870	7.4	(c) 118	84	69	191	2.77	8,140	22.6
																(b) 107	79	48	75	1.56	5,140	8.0	(d) 141	84	82	84	1.02	11,582	11.8
																							(e) 134	89	69	118	1.71	9,242	15.8
	1–29	(a) 75	70	56	92	1.63	4,200	6.9	(b) 90	84	27	48	1.78	2,430	4.3														
	2– 6	(a) 65.4	69	45	74	1.64	2,950	4.8	(b) 96	86	25	41	1.64	2,400	3.9														
	2–13	(b) 66.3†	73	43	54	1.26	2,850	3.6	(a) 96	89*	26	33	1.28	2,500	3.2														
									(c) 91	78*	24	33	1.38	2,180	3.0														
	2–20	(a) 69	76	40	84	2.1	2,760	5.8	(b) 89	99	26	44	1.69	2,310	3.9														
Average ..		72.1	72.5	45.3	82	1.79	3,279	5.85	95.5	86.9	25.3	39.5	1.56	2,471	3.72	108	79.3	46.6	78	1.66	5,063	8.4	131	86	73	131	1.83	9,882	16.7
W.D.	12–15	(a) 80†	71	37	49	1.32	2,960	3.9	(b) 94†	67	31	41	1.32	2,910	3.9														
	12–22															(a) 85	63	55	99	1.79	4,346	8.4	(b) 121†	76	56	90	1.61	7,017	10.9
	1–12	(a) 72	63	44	122	2.77	3,170	8.8	(b) 88†	71	31	78	2.51	2,730	6.9														
	1–13															(a) 84	63	51	93	1.82	4,280	7.8	(b) 128	64	85	146	1.72	10,090	18.7
	3– 3	(b) 60.3	64	44	104	2.35	2,875	6.3	(a) 78	73*	34	51	1.50	2,580	4.0														
	3–20	(a) 64	59	47	123	2.66	2,970	7.5	(b) 82	71	34	50	1.47	2,790	4.1														
		(c) 65	62	45	89	1.96	2,960	5.8	(d) 86	73	34	65	1.91	2,920	5.5														
	3–30								(a) 84	71*	30	60	2.0	2,520	5.0														
		(c) 62	63	43	101	2.35	2,670	6.6	(b) 84	68*	33	64	1.94	2,770	5.3														
Average...		64.6	62.2	45.6	108	2.42	2,889	7.06	82.8	71.8	33	58	1.77	2,716	4.78	84.5	63	53	96	1.805	4,313	8.1							

E. E.	1–22	(a) 65	84	46	92	2.00	2,950	6.0	(c) 97	91*	31	41	1.33	2,974	4.0											
		(b) 64	88	41	80	1.95	2,624	5.1	(d) 98	86*	37	54	1.46	3,647	5.3											
	1–23															(a) 90	88	48	79	1.64	4,320	7.1				
																(b) 86	97	48	73	1.52	4,150	6.3				
	1–26																						(c) 95	87	56	92	1.55	5,320	8.7
																							(d) 92	87	71	115	1.62	6,520	10.6
	3–18								(a) 86	94	32	45	1.41	2,750	3.9											
									(b) 93	83	33	44	1.34	3,060	4.1											
Average...		64.5	86	43.5	86	1.97	2,785	5.5	93.5	88.5	33.2	46	1.38	3,108	4.32	88	92.5	48	76	1.58	4,235	6.7	93	87	64	103	1.58	5,820	9.65
D. W.	1–27	(a) 70	72	41	114	2.78	2,870	8.0																				
		(b) 68	72	40	96	2.4	2,720	6.5																				
	1–25															(a) 106	74	57	102	1.45	6,050	10.8						
																(b) 101	70	58	84	1.79	5,870	8.5						
	1–28								(a) 96†	90	32	31	0.97	2,980	2.9								(a) 120	69	90	131	1.46	10,800	15.8
									(b) 95†	84	29	28	0.97	2,755	2.6								(b) 114	73	81	127	1.57	9,250	14.5
	3–22								(a) 101	75	35	51	1.46	3,465	5.2													
									(b) 99	72	35	45	1.29	3,580	4.4													
Average...		69	72	40.5	105	2.59	2,795	7.25	100	73.5	35	48	1.37	3,523	4.8	103.5	72	57.5	93	1.62	5,960	9.7	117	71	85	129	1.51	10,025	15.1

P.R. = pulse rate.
D.P. = diastolic pressure.
P.P. = pulse pressure.
S.V. = stroke volume.
C.R. = circulation rate.
* Diastolic pressures difficult to read.
† Excluded from averages

the subject was ill; he had fever and, as will be observed, a relatively rapid pulse rate; 2, D. W., 1/28, standing, because the subject became faint while these determinations were being made; and 3, W. D., 12/22, heavy exercise, because the determinations were carried out while the subject was in the standing instead of the recumbent posture.

After the exclusion of the above-mentioned determinations, there are left in the case of T. H. six satisfactory determinations of the circulation rate in the recumbent posture, of which five may be regarded as comparable in that the ranges of the pulse rate (77 to 65.4), of the diastolic pressure (69 to 77), and of the pulse pressure (40 to 45), are fairly narrow. The stroke volume range here is 74 to 84 cc. If this variation were due entirely to error, which of course is not the case, this would amount to only ± 7 per cent.

Eight determinations were made on the same subject while standing, but owing to the wide range of the pulse rate (110 to 88) and of the diastolic pressure (99 to 78) comparison of these determinations one with the other is scarcely justifiable. If the entire variation in stroke volume were chargeable to error in the determination of the circulation rate, a conclusion which cannot of course be defended, the error would amount to $+22$ -16 per cent.

In the case of W. D., recumbent, there are four sets of observations (3/3, 3/20, bis, 3/30) in which the ranges of pulse rate (60 to 65), diastolic pressure (59 to 64) and pulse pressure (47 to 43) are reasonably narrow. The stroke volume, however, ranges between 89 and 123, or $+18$ -14 per cent. There is in this case no way out of charging a considerable part of this fluctuation against error in the determination of the circulation rate. The observations made on W. D., while standing, exhibit a better agreement. The ranges of the pulse rate, diastolic pressure, and pulse pressure were narrow (78 to 86, 68 to 73, and 30 to 34 respectively). The range in the systolic volume was 50 to 65, making the maximum experimental error in the determination of the circulation rate ± 15 per cent. In the case of the remainder of the subjects there is not a sufficient number of determinations to justify analysis by this method; it is evident, however, on a basis of the data available that there may be considerable error in the determination of the circulation rate. At the outside, however, and excluding gross errors which duplicate determinations would easily discern, it cannot be greater than about ± 15 per cent and probably is considerably less than this figure.

If there be between the stroke volume and the pulse pressure a relation of direct proportionality, these two sets of data when plotted against each other in a system of rectangular coördinates should fall on a straight line passing through zero. Fluctuations from linearity might find their explanation in 1, experimental error; 2, variations in the elasticity of the

arteries; 3, variations in systolic time, and 4, variations in the capacity of the arterial reservoir. 1. Experimental error will result merely in an irregular scattering of the points to either side of the line; it can be minimized, as has been said, by averaging a sufficient number of comparable determinations. 2. The elasticity of the arteries in all probability depends upon the arterial pressure. This relation has been worked out in man by Bramwell, Downing and Hill (1923). Their curve shows that it is just in the range of our diastolic pressures that the coefficient of elasticity of the arteries changes most rapidly with changing pressures. The higher the diastolic pressure, therefore, the larger would be the pulse pressure produced by a given stroke volume. Since our data include determinations of the diastolic pressure it should be possible to obtain some idea with regard to the influence of this factor. 3. The shorter the systolic time the larger will be the pulse pressure produced by a given stroke volume. We have not determined the systolic time but it is fair to assume that in general the systolic time is shorter the more rapid the pulse rate. Katz and Feil (1923) have reviewed the literature on this subject. It is possible, therefore, that in an analysis of the character of the deviations from linearity, some account can be taken of this factor also. 4. Whether the arterial tree is subject to changes in volume independently of those due to pressure variations is questionable. However this may be, means of taking any such variations into account are not available.

All of the observations on stroke volume and pulse pressure without exception are plotted in figure 1, but those excluded in computing the average values of the various states are indicated by the underscored numbers, and those excluded because of obvious error, by the double underscoring. Examination of these graphs shows that there is a very decided tendency for the points to collect in groups. The group nearest zero includes all of the data on "standing" (circles). The middle group includes practically all of the "recumbent" observations (hollow squares) and also the "light exercise" observations (dots). Then there is seen a tendency for the points representing "heavy exercise" (solid squares) to fall still further out. When each group is examined individually, even if the underscored observations be excluded, it is seen that the points do not fall on a straight line. This scattering of the points unquestionably is the expression of the experimental error.

The positions taken by the averages of the determinations of each of the groups are indicated by the crosses. In all four cases the lines joining the crosses representing standing and light exercise almost or actually pass through zero and through the cross representing heavy exercise. On the other hand, the mean of the recumbent readings always falls somewhat below this line. The explanation of this deviation undoubtedly is to be found in the effect the arterial pressure and the systolic time have upon the

The Relation of Pulse Pressure to Stroke Volume plotted for each Subject.

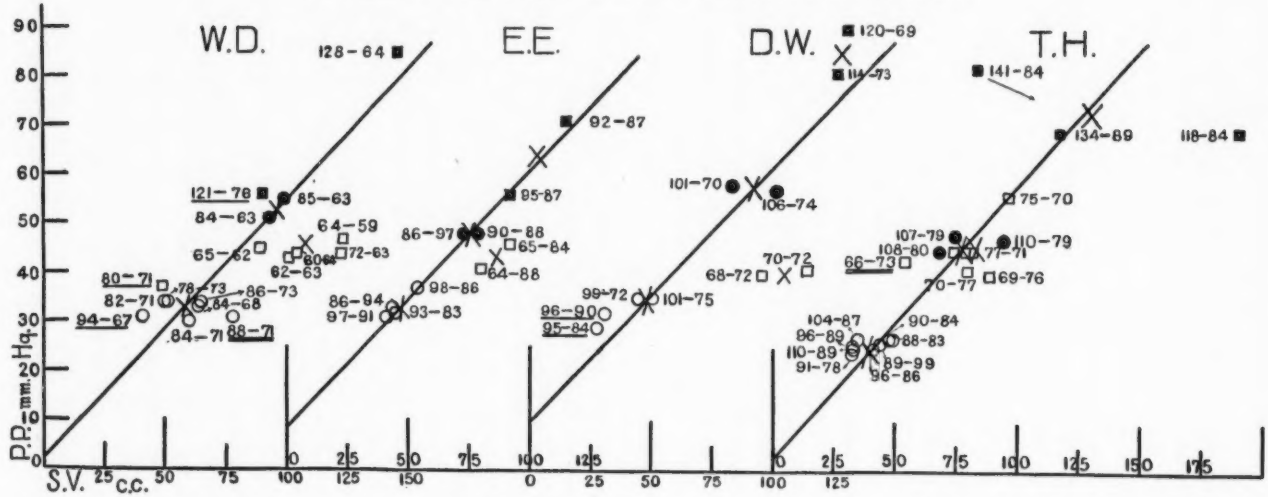

Fig. 1. □ Recumbent. ● Light exercise. ○ Standing. ■ Post-heavy exercise. × Mean values. The first of the two figures adjoining each plotted point is the pulse rate; the second, the diastolic pressure.

relation of the pulse pressure to the systolic volume. Examination of the data shows that the "recumbent" values of the diastolic pressure are either the same as or lower than the diastolic determinations in the other states. The effect of the lower diastolic pressure would be to reduce the pulse pressure produced by a given stroke volume; and this would tend to throw the "recumbent" point below the "standing-exercise" line. This relation is so constant that it cannot be attributed to chance.

That the line joining the "standing" and the "exercise" points sometimes fails to pass through zero is not surprising; for the positions of these points also must be influenced by the associated diastolic pressures and systolic times. It must be due merely to the circumstance that "standing" and "light exercise" in most of the subjects, at least, develop comparable diastolic pressures and pulse rates; it is this coincidence that makes the pulse pressure in these two states almost proportional to the stroke volume. That in each case the "heavy exercise" point almost exactly falls on the "standing-light exercise" line, considering the small number of determinations and the changing conditions under which they were made, again must be attributed in part to chance: but it cannot be wholly a matter of chance that in every one of the four cases the agreement with proportionality is so close.

Presumably it is the modifying influence of the arterial pressure upon the relation of the pulse pressure to the stroke volume that accounts for the position (above the line) taken by the two lowest points in the chart of D. W. The data upon which these two points are based were obtained, it will be recalled, while the subject felt faint. The diastolic pressures obtaining in these instances are by far the highest of the series; the pulse rate also is relatively rapid. The high diastolic pressure will have the effect of causing the pulse pressure to be relatively large for the stroke volume, thus causing the plotted points to fall above the line, as they do.

Direct proportionality requires that in any given subject the ratio $\frac{SV}{PP}$ be constant. The range of the variation of this ratio in each of the four subjects, disregarding the questioned observations, has been 170 per cent, 88 per cent, 50 per cent, and 115 per cent, respectively. If, in order to minimize the experimental error one takes, instead, the mean values of the four states especially studied, the ratios in each of the four subjects become 17 per cent, 41 per cent, 43 per cent and 89 per cent. These variations, it should be noted, are very much narrower than those occurring in the two animal experiments performed by Miss Skelton, namely, 254 and 572 per cent, respectively.

We may now examine the data with a view to ascertaining in how far the alteration from direct proportionality resulting from changing elasticity of the arteries and changing systolic time will interfere with the unqualified

use in one and the same subject of the product P.P. × P.R. as an index to the circulation rate, both absolutely and in consecutive determinations. Considering first average values, so as to eliminate experimental error as far as possible, when the stroke volume-pulse pressure ratios of each subject while standing are compared with those obtained during light exercise, it is found that without exception the latter are slightly larger than the former. The differences, however, are not very great. Without going into detail it will suffice to say that the changes in pulse rate and diastolic pressure are such as to account for the changes in the ratio. Again when the ratios of the "standing" values are compared with those of the "post-heavy exercise" values it is found that the latter are larger than the former; and where the differences are considerable they again can be accounted for readily on the basis of the effect the increased pulse rate occurring during heavy exercise has upon the systolic time. As has been pointed out, the ratios stroke volume-pulse pressure obtained in the recumbent position are entirely too large relative to those obtaining in the other states, due to the effect upon the pulse pressure of the low diastolic pressure and the slow pulse rate obtaining in the former.

The table shows, however, that despite the large experimental error and despite differences from observation to observation in pulse rate and arterial pressure there are only four instances where in successive determinations (sequence is indicated by letters) the circulation rate fails to change in the same direction as the product P.P. × P.R. These cases may be examined individually.

1. T. H., 1/15, heavy exercise. Here determination d has the largest product but the smallest circulation rate of three successive determinations. This possibly is due to the fact that in these experiments the period during which the circulation was determined did not exactly coincide with the period of the determination of the arterial pressure. Since these determinations are carried out at a time when a very rapid readjustment of the circulation rate is in progress such a slight time difference might cause some discordance. But it is also possible that the discrepancy here is a manifestation of the difference in the pulse rate, the more rapid rate obtaining in d producing a pulse pressure that is too high relative to the stroke volume.

2. D. W., 3/22, standing. Here in two consecutive determinations P.P. × P.R. increases from 3465 to 3580 while the circulation rate decreases from 5.2 to 4.4 liters. The cause for this discrepancy is not apparent. It is no greater, however, than the experimental error of the determination of the pulse pressure and of the circulation rate.

3. W. D., 3/30. The change from standing, b, to recumbent, c, postures changes the product P.P. × P.R. from 2770 to 2670 while the circulation rate increases from 5.3 to 6.6 liters. These changes, likewise, fall

within the experimental error, but even were there no error, the effect of the slower pulse rate in c, owing to the associated longer systolic time, would be sufficient to thus disturb the linear relation.

4. E. E., 1/22 (b to c). This case is much like 3 except that the deviation here is greater than can be explained on the basis of experimental error and undoubtedly is referable to the difference in systolic times.

When the average values of the four different states are compared it is found that with but two exceptions the product of P.P. $\times$ P.R. varies in the same direction as the circulation rate. In the case of E. E. the standing-recumbent ratio of the product P.P. $\times$ P.R. is $\frac{3108}{2785}$ and of the circulation rates $\frac{4.3}{5.5}$, and in D. W. $\frac{3523}{2795}$ and $\frac{4.8}{7.2}$ respectively. Now it will be noted that the decrease in pulse rate that occurs with this change in posture is much greater in the case of these two subjects (E. E. 29, D. W. 31) than in the case of the others (T. H. 23, W. D. 22), and it, undoubtedly, is the effect that the systolic time has upon the pulse pressure that causes here the opposite variation of P.P. $\times$ P.R. and circulation rate.

It is thus demonstrated that the relation of the product P.P. $\times$ P.R. to the circulation rate undoubtedly is one of direct proportionality which is altered somewhat in individual determinations by experimental error, by differences in the extensibility of the arteries, and by differences in the systolic time. Practically, as is indicated by the 58 determinations we have made, these disturbing factors will rarely have the effect of obscuring this proportionality. But the possibility that they may, must always be borne in mind when the product P.P. $\times$ P.R. is being employed to indicate the direction of consecutive changes in the circulation rate. There can be no difficulty, except that due to experimental error, where in the consecutive readings the diastolic pressures and pulse rates are nearly alike. And when these are not alike one can usually ascertain from the directions of their changes whether the changes in the circulation rate indicated by the product P.P. $\times$ P.R. are too large or too small and in this way be on one's guard against misinterpretation of the data.

In any series of observations in which accurate data on the pulse pressure are needed it obviously is essential, therefore, to reduce the experimental error of its determination to a minimum. Our experience indicates that this can best be done by making, as objectively as possible, simultaneous readings by two methods and using one set of readings as a check upon the other.

It is scarcely necessary to point out that our data confirm Field and Bock (1925) in showing that the circulation rate is greater in the recumbent than in the standing position. This finding is rather surprising in view of the fact that the metabolic rate is higher while the subject is standing. Indeed

the circulation rate obtaining in the recumbent position may be as great as when the subject is taking light exercise. The significance from the teleological standpoint, of the rapid circulation rate in the recumbent position is not obvious. In casting about for a rational explanation the results obtained by Shepard (1914) in his study of the cerebral circulation seemed suggestive. Shepard concluded from this study that the brain is liberally supplied with blood during sleep, whereas during the waking hours the cerebral vessels are maximally constricted. During mental effort under such circumstances the blood supply to the brain increases, the increase being effected through a shunting of blood into the brain by a vasoconstriction in the rest of the body. Now it has occurred to us that during rest in the recumbent posture the vascular relaxation might involve not only

TABLE 2

Subject T. H.

	CIRCULATION RATE, LITERS PER MINUTE	Q STROKE VOLUME IN LITERS	ARTERIAL RESISTANCE IN MM.Hg D.P. + ⅓ P.P.	R ARTERIAL RESISTANCE IN METERS OF BLOOD M.P. × 0.013	V MEAN VELOCITY IN METERS PER SECOND	PULSE RATE	C CARDIAC CYCLE IN SECONDS	E SYSTOLIC TIME IN SECONDS	W WORK IN KILOGRAM-METERS PER MINUTE	WORK IN KILOGRAM-METERS PER STROKE	WORK IN KILOGRAM-METERS PER LITER OF BLOOD	PER CENT OF TOTAL WORK REPRESENTED BY VELOCITY FACTOR
Recumbent....	5.85	0.082	87.6	1.19	0.20	72.0	0.83	0.31	8.23	0.116	1.42	1.9
Standing.....	3.7	0.04	95.3	1.295	0.13	95.5	0.63	0.24	5.66	0.06	1 53	0.8
Light exercise..	8.4	0.078	94.8	1.29	0.28	108.0	0.56	0.21	13.2	0.122	1.57	3.6
Post heavy exercise.....	16.7	0.131	110.3	1.50	0.57	131.0	0.46	0.23	31.4	0.24	1.88	7.0

the brain, but all other parts as well, and might account for the rapid circulation; and that during attention, and therefore while standing, there is a general vasoconstriction, perhaps over and above that needed to compensate the effect of gravity upon the circulation, and a slowed circulation, the general constriction giving way to a selective dilatation in localities actively functioning.

WORK OF THE HEART. The data from our experiments afford most of the factors needed for the calculation of the work done by the heart per minute, per stroke, and for the expulsion of a liter of blood. The corrected formula of Evans (1918) $W = \frac{7QR}{6} + \frac{w(VC)^2}{gE^2}$ has been used, where W = work in kilogram-meters per minute; Q = volume of blood expelled in

liters per minute; R = mean arterial resistance in meters of blood (diastolic pressure + $\frac{1}{3}$ pulse pressure in mm. Hg, and the sum multiplied by 0.013); V = mean velocity of blood at the root of the aorta; g = gravity constant expressed in meters per second; C = time of cardiac cycle in seconds; E = systolic time in seconds. W divided by the pulse rate equals the work done per stroke, and when divided by the circulation rate equals the work done in expelling a liter of blood. We have assumed that in the recumbent, standing, and light exercise states, the systolic time occupies three-eighths of the cardiac cycle, while with heavy exercise the systolic time is one-half of the cardiac cycle. We have also assumed for our calculation of mean velocity, as did Evans, that the diameter of the aortic orifice is 2.5 cm., or 5 sq. cm. in cross section area.

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SUMMARY

Simultaneous determination in man of the circulation rate by the ethyl iodide method and of arterial pressure show that within the limit of error the pulse pressure is directly proportional to the stroke volume under conditions that have essentially the same diastolic pressure and pulse rate. When variations from proportionality occur, they can be attributed to the effect that differences in the coefficient of elasticity of the arterial wall, determined by the arterial pressure, and that differences in pulse rate, and consequently in systolic time, have upon the relation of the stroke volume to the pulse pressure. By taking this into consideration it should be possible to employ the product of the pulse pressure and the pulse rate as an index to the circulation rate in consecutive observations in one and the same subject.

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THE INFLUENCE OF POSTURE ON RENAL ACTIVI

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From the Physiological Department of Washington University, St. Lo

Received for publication May 17, 1926

Two old and fundamental questions of renal physiology which s
the attention of workers in this field are, "What are the factor
determine the passage of fluid across the glomerular membrane
"Do the tubular cells add anything to the glomerular fluid?" No
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tions. We shall first consider the bearing of our experiments on
of these questions. Richards and Plant (1922) present a brief re
the old evidence. They conclude from their own experiments t
rate of urine formation is a function primarily of the pressure in
merular capillaries. It has long been known (Edel, 1901) that the
of urine passed is greater in the recumbent than in the standing p
Erlanger and Hooker (1904) found that the changes in volume
passed on changing posture were related to changes in pulse press
not to changes in either systolic or diastolic pressures except in s
these affect pulse pressure.

In the experiments reported here the influence of posture on th
lation rate, pulse rate and output of various urinary constituents,
as on the blood pressure and volume of urine, were followed. Nine
ments were performed on three subjects, four on subject H., age 1
on subject D., age 30 and two on subject S., age 50. The proce
an experiment was as follows. The subject took no food or wat
10:00 p.m. on the evening preceding the day of the experime
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He then stood or lay for two hours, voiding at the end of each tw
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Each experiment was continued for four consecutive two-hour
The circulation rate for a ten-minute period was determined by He
and Haggard's method (1925) in at least one lying and one standing
During the period of circulation rate determination ten arterial blo
sure readings were made, using the auscultatory and graphic (F

POST	TIME	cc.	SPEC.	CO_2	NaCl	CREA.	P. M.	S. M.	S. BR.	DIUR.	CREA.	TITR.	pH	STRIC.	PCTSN.	PCTSN.	CHOL.	BOOK
S.	8–10	28	1.029	3.8	389	1,687	472	76.4	21.4	98.6	27.6	133.0	37.3	193.0	54.0	52.0	14.5	5.2	124	94	30	95		24.5
R.	10–12	115	1.010	10.8	637	369	424	11.7	13.5	18.5	21.3	23.3	26.8	44.0	50.6	7.5	8.6	6.0	113	71	42	75	4	24.5
S.	12– 2	59	1.011	10.0	328	244	25.0	14.7	24.6	14.5	40.8	24.1	102.0	60.4	11.0	6.5	5.6	113	87	26	97	3	24.0
R.	2– 4	152	1.006	9.4	288	249	379	13.1	19.9	10.2	15.5	14.3	21.7	37.5	57.0	7.0	10.6	5.9						24.0

[1] Fellow in Medicine of the National Research Council.

the circulation rate obtaining in the recumbent position may be as great as when the subject is taking light exercise. The significance from the teleological standpoint, of the rapid circulation rate in the recumbent position is not obvious. In casting about for a rational explanation the results obtained by Shepard (1914) in his study of the cerebral circulation seemed suggestive. Shepard concluded from this study that the brain is liberally supplied with blood during sleep, whereas during the waking hours the cerebral vessels are maximally constricted. During mental effort under such circumstances the blood supply to the brain increases, the increase being effected through a shunting of blood into the brain by a vasoconstriction in the rest of the body. Now it has occurred to us that during rest in the recumbent posture the vascular relaxation might involve not only

TABLE 2

Subject T. H.

	CIRCULATION RATE, LITERS PER MINUTE	Q STROKE VOLUME IN LITERS	ARTERIAL RESISTANCE IN MM.Hg D.P. + ½ P.P.	R ARTERIAL RESISTANCE IN METERS OF BLOOD M.P. × 0.013	V MEAN VELOCITY IN METERS PER SECOND	PULSE RATE	C CARDIAC CYCLE IN SECONDS	E SYSTOLIC TIME IN SECONDS	W WORK IN KILOGRAM-METERS PER MINUTE	WORK IN KILOGRAM-METERS PER STROKE	WORK IN KILOGRAM-METERS PER LITER OF BLOOD	PER CENT OF TOTAL WORK REPRESENTED BY VELOCITY FACTOR
Recumbent....	5.85	0.082	87.6	1.19	0.20	72.0	0.83	0.31	8.23	0.116	1.42	1.9
Standing.....	3.7	0.04	95.3	1.295	0.13	95.5	0.63	0.24	5.66	0.06	1 53	0 8
Light exercise..	8.4	0.078	94.8	1.29	0.28	108.0	0.56	0.21	13.2	0.122	1.57	3.6
Post heavy exercise.....	16.7	0.131	110.3	1.50	0.57	131.0	0.46	0.23	31.4	0.24	1.88	7.0

the brain, but all other parts as well, and might account for the rapid circulation; and that during attention, and therefore while standing, there is a general vasoconstriction, perhaps over and above that needed to compensate the effect of gravity upon the circulation, and a slowed circulation, the general constriction giving way to a selective dilatation in localities actively functioning.

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In the experiments reported here the influence of posture on the circulation rate, pulse rate and output of various urinary constituents, as well as on the blood pressure and volume of urine, were followed. Nine experiments were performed on three subjects, four on subject H., age 18, three on subject D., age 30 and two on subject S., age 50. The procedure for an experiment was as follows. The subject took no food or water after 10:00 p.m. on the evening preceding the day of the experiment. At 8:00 a.m. he drank 200 cc. of tap water, voided and discarded the urine. He then stood or lay for two hours, voiding at the end of each two hour period, and drank 200 cc. of water at the beginning of each period. The order of lying and standing periods was varied in the various experiments. Each experiment was continued for four consecutive two-hour periods. The circulation rate for a ten-minute period was determined by Henderson and Haggard's method (1925) in at least one lying and one standing period. During the period of circulation rate determination ten arterial blood pressure readings were made, using the auscultatory and graphic (Erlanger

[1] Fellow in Medicine of the National Research Council.

TABLE 1

Subject H. February 6, 1926

POSTURE	TIME	CC. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 CC.	CO_2, MGM. PER HOUR	NaCl, MGM. PER 100 CC.	NaCl, MGM. PER HOUR	UREA N, MGM. PER 100 CC.	UREA N, MGM. PER HOUR	P, MGM. PER 100 CC.	P, MGM. PER HOUR	S, MGM. PER 100 CC.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 CC.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 CC.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, CC. $\frac{N}{10}$ PER 100 CC.	TITRATABLE ACID, CC. $\frac{N}{10}$ PER HOUR	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE
S.	8–10	36	1.025	4.6	1.6	1,336	481	742	267	57.1	20.5	72.0	25.9	91.0	33.7	147	53.0	25	9.0					
R.	10–12	208	1.011	3.3	6.9	448	932	195	406	16.6	34.5	12.4	25.8	17.3	35.9	29.1	60.5	7	14.6	114	69	45	65.4	4.8
R.	12– 2	284	1.012	11.0	31.2	577	1,640	175	497	13.3	37.8	10.2	29.0	10.0	28.4	20.5	58.2	2	5.7					
S.	2– 4	61	1.013	14.2	8.7	568	347	332	203	33.3	20.3	27.7	16.9	38.8	23.7	81.8	50.0	9	5.5	111	86	25	96.0	3.9

TABLE 2

Subject H. February 13, 1926

POSTURE	TIME	CC. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 CC.	CO_2, MGM. PER HOUR	NaCl, MGM. PER 100 CC.	NaCl, MGM. PER HOUR	UREA N, MGM. PER 100 CC.	UREA N, MGM. PER HOUR	P, MGM. PER 100 CC.	P, MGM. PER HOUR	S, MGM. PER 100 CC.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 CC.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 CC.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, CC. $\frac{N}{10}$ PER 100 CC.	TITRATABLE ACID, CC. $\frac{N}{10}$ PER HOUR	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE
R.	8–10	235	1.012	15.6	36.6	679	1,590	263	619	9.4	22.1	17.0	40.0	14.5	34.1	29.1	68.3	2.2	5.2					
S.	10–12	54	1.019	10.5	5.6	1,124	608	473	256	14.3	7.7	50.0	27.0	58.8	31.8	95.3	51.4	4.5	2.4	118	90	28	96.0	3.2
R.	12– 2	175	1.006	6.4	11.2	318	556	253	444	10.5	18.4	17.1	29.9	20.9	35.5	33.0	57.7	3.0	5.2	115	73	42	66.3	3.6
S.	2– 4	68	1.010	3.0	2.0	496	337	367	249	22.0	15.0	30.0	20.4	41.7	28.4	78.0	53.0	6.4	4.3	110	80	30	90.9	3.0

TABLE 3

Subject H. February 20, 1926

TABLE 3

Subject H. February 20, 1926

POSTURE	TIME	cc. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 cc.	CO_2, MGM. PER HOUR	NaCl, MGM. PER 100 cc.	NaCl, MGM. PER HOUR	UREA N, MGM. PER 100 cc.	UREA N, MGM. PER HOUR	P, MGM. PER 100 cc.	P, MGM. PER HOUR	S, MGM. PER 100 cc.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 cc.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 cc.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, cc. $\frac{N}{10}$ PER 100 cc.	TITRATABLE ACID, cc. $\frac{N}{10}$ PER HOUR	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE	ROOM TEMPERATURE
R.	8–10	180	1.008	96.0	173.0	522	940	218	392	6.5	11.7	14.9	26.8	4.6	8.3	39.7	71.3	alk.	alk.	116	76	40	69	5.8	20.5
S.	10–12	55	1.009	28.8	15.8	396	218	317	174	8.6	4.7	21.5	11.8	27.8	15.3	122.0	67.2	1.5	0.8	125	99	26	89	3.9	21.0
R.	12– 2	129	1.007	11.5	14.8	297	383	387	498	14.9	19.2	17.3	22.3	27.8	35.9	53.2	68.6	5.0	6.5						21.5
S.	2– 4	47	1.012	7.7	3.6	406	191	502	236	31.5	14.8	26.7	12.6	53.6	25.2	117.0	54.8	13.0	6.1						22.0

TABLE 4

Subject H. February 27, 1926

POSTURE	TIME	cc. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 cc.	CO_2, MGM. PER HOUR	NaCl, MGM. PER 100 cc.	NaCl, MGM. PER HOUR	UREA N, MGM. PER 100 cc.	UREA N, MGM. PER HOUR	P, MGM. PER 100 cc.	P, MGM. PER HOUR	S, MGM. PER 100 cc.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 cc.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 cc.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, cc. $\frac{N}{10}$ PER 100 cc.	TITRATABLE ACID, cc. $\frac{N}{10}$ PER HOUR	pH	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE	ROOM TEMPERATURE
S.	8–10	28	1.029	3.8	1.1	1,390	389	1,687	472	76.4	21.4	98.6	27.6	133.0	37.3	193.0	54.0	52.0	14.5	5.2	124	94	30	95		24.5
R.	10–12	115	1.010	10.8	12.4	554	637	369	424	11.7	13.5	18.5	21.3	23.3	26.8	44.0	50.6	7.5	8.6	6.0	113	71	42	75	4.4	24.5
S.	12– 2	59	1.011	10.0	5.9	555	328	414	244	25.0	14.7	24.6	14.5	40.8	24.1	102.0	60.4	11.0	6.5	5.6	113	87	26	97	3.9	24.0
R.	2– 4	152	1.006	9.4	14.3	196	298	249	379	13.1	19.9	10.2	15.5	14.3	21.7	37.5	57.0	7.0	10.6	5.9						24.0

TABLE 5

Subject D. March 3, 1926

POSTURE	TIME	CC. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 CC.	CO_2, MGM. PER HOUR	NaCl, MGM. PER 100 CC.	NaCl, MGM. PER HOUR	UREA N, MGM. PER 100 CC.	UREA N, MGM. PER HOUR	P_i MGM. PER 100 CC.	P_i MGM. PER HOUR	S, MGM. PER 100 CC.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 CC.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 CC.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, CC. $\frac{N}{10}$ PER 100 CC.	TITRATABLE ACID, CC. $\frac{N}{10}$ PER HOUR	pH	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE	ROOM TEMPERATURE
S.	8–10	41	1.018	4.9	2.0	1,024	420	885	363	29.6	12.1	55.6	22.8	69.0	28.3	123.0	50.4	20.0	8.2	5.1						23.5
R.	10–12	168	1.005	3.6	12.1	354	594	316	530	14.6	24.5	13.6	22.9	18.2	30.6	30.7	51.6	9.0	15.1	4.7	103	62	41	62		23.5
S.	12– 2	116	1.007	3.9	4.5	371	431	339	394	17.5	20.3	14.0	16.3	20.8	24.2	47.0	54.4	11.5	13.3	4.9	107	73	34	78	4.0	23.5
R.	2– 4	143	1.008	5.0	7.2	399	570	258	369	19.1	27.3	13.8	19.7	20.0	28.6	40.2	57.5	10.0	14.3	5.1	108	64	44	60	6.3	23.5

TABLE 6

Subject D. March 20, 1926

POSTURE	TIME	CC. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 CC.	CO_2, MGM. PER HOUR	NaCl, MGM. PER 100 CC.	NaCl, MGM. PER HOUR	UREA N, MGM. PER 100 CC.	UREA N, MGM. PER HOUR	P_i, MGM. PER 100 CC.	P_i, MGM. PER HOUR	S, MGM. PER 100 CC.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 CC.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 CC.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, CC. $\frac{N}{10}$ PER 100 CC.	TITRATABLE ACID, CC. $\frac{N}{10}$ PER HOUR	pH	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE	ROOM TEMPERATURE
R.	7– 9	47	1.020	8.7	4.1	880	414	950	446	32.5	15.3	53.6	25.2	50.0	23.5	101.0	47.5	16	7.5	5.5	106	59	47	64	7.9	24.8
S.	9–11	25	1.023	5.8	1.5	844	211	1,640	410	41.6	10.4	79.0	19.7	100.0	25.0	186.0	46.5	24	6.0	5.3	106	71	35	82	4.1	25.0
R.	11– 1	115	1.010	3.8	4.4	469	540	523	603	24.6	28.3	21.1	24.3	33.3	38.3	42.3	48.7	13	15.0	5.1	107	62	45	65	5.8	25.0
S.	1– 3	74	1.011	3.6	2.7	560	414	854	632	29.3	21.7	26.0	19.2	35.7	26.4	54.1	40.0	18	13.0	4 9	108	73	34	86	5.6	24.8

TABLE 7

Subject D. March 29, 1926

R.	11- 1	115	1.015	5.3	4.4	409	340	525	603	24.6	28.3	21.1	24.3	33.3	38.3	42.3	48.7	13	15.0	5.1	107	62	45	65	5.8	25.0
S.	1- 3	74	1.011	3.6	2.7	560	414	854	632	29.3	21.7	26.0	19.2	35.7	26.4	54.1	40.0	18	13.0	4 9	108	73	34	86	5.6	24.8

TABLE 7

Subject D. March 29, 1926

POSTURE	TIME	CC. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 CC.	CO_2, MGM. PER HOUR	NaCl, MGM. PER 100 CC.	NaCl, MGM. PER HOUR	UREA N, MGM. PER 100 CC.	UREA N, MGM. PER HOUR	P, MGM. PER 100 CC.	P, MGM. PER HOUR	S, MGM. PER 100 CC.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 CC.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 CC.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, CC. $\frac{N}{10}$ PER 100 CC.	TITRATABLE ACID, CC. $\frac{N}{10}$ PER HOUR	pH	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE	ROOM TEMPERATURE
S.	7- 9	11.7		4.8	0.6	1,000	117	1,508	177	58.8	6.9	130.0	15.2	159.0	18.6	411.0	48.1	42.4	5.0	5.0	101	71	30	84	5.0	23.0
R.	9-11	46.0	1.015	6.0	2.8	340	156	1,033	475	26.3	12.1	44.5	20.5	46.5	21.4	119.0	54.8	20.6	9.5	5.3						23.5
S.	11- 1	34.0	1.016	4.6	1.6	542	184	1,002	341	44.5	15.1	48.8	16.6	108.0	36.8	137.0	46.7	30.0	10.2	4.8	101	68	33	84	5.3	23.9
R.	1- 3	96.0	1.011	4.0	3.8	554	532	525	504	24.0	23.0	24.2	23.2	30.6	29.4	58.8	56.5	16.0	15.4	4.7	106	63	43	62	6.6	24.0

TABLE 8

Subject S. February 23, 1926

POSTURE	TIME	CC. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 CC.	CO_2, MGM. PER HOUR	NaCl, MGM. PER 100 CC.	NaCl, MGM. PER HOUR	UREA N, MGM. PER 100 CC.	UREA N, MGM. PER HOUR	P, MGM. PER 100 CC.	P, MGM. PER HOUR	S, MGM. PER 100 CC.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 CC.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 CC.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, CC. $\frac{N}{10}$ PER 100 CC.	TITRATABLE ACID, CC. $\frac{N}{10}$ PER HOUR	pH	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE	ROOM TEMPERATURE
R.	8-10	330	1.003	38.0	125.0	383	1,264	125	412	10.0	33.0	7.8	25.8	3.3	10.9	16.2	53.6	0.5	1.7	6.8						25.5
S.	10-12	52	1.010	16.5	8.6	650	338	360	187	35.7	18.6	24.5	12.8	16.7	8.7	95.3	49.6	9.0	4.7	5.9	158	103	55	96	4.0	25.5
R.	12- 2	120	1.004	11.0	13.2	391	469	217	260	23.8	28.6	11.1	13.3	10.4	12.5	38.6	46.3	8.5	10.2	5.8	171	104	67	85	6.5	26.0
S.	2- 4	45	1.013	7.2	3.2	500	225	389	175	62.6	28.2	26.2	11.8	27.8	12.5	108.0	48.6	17.0	7.7	5.0						25.5

TABLE 9

Subject S. March 24, 1926

PERIOD	TIME	cc. URINE PER HOUR	SPECIFIC GRAVITY	CO_2, MGM. PER 100 cc.	CO_2, MGM. PER HOUR	$NaCl$, MGM. PER 100 cc.	$NaCl$, MGM. PER HOUR	UREA N, MGM. PER 100 cc.	UREA N, MGM. PER HOUR	P, MGM. PER 100 cc.	P, MGM. PER HOUR	S, MGM. PER 100 cc.	S, MGM. PER HOUR	AMMONIA N, MGM. PER 100 cc.	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER 100 cc.	CREATININE, MGM. PER HOUR	TITRATABLE ACID, cc. $\frac{N}{10}$ PER 100 cc.	TITRATABLE ACID, cc. $\frac{N}{10}$ PER HOUR	pH	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE	CIRCULATION RATE, LITERS PER MINUTE	ROOM TEMPERATURE
S.	8–10	18.5	1.026	9.0	1.7	1,124	208	844	156	60.6	11.2	55.4	10.2	95.2	17.6	188.0	34.7	28.0	5.2	6.0	139	102	37	86	3.8	23.8
R.	10–12	109.0	1.005	8.8	9.6	322	351	285	311	19.7	21.5	10.0	10.9	9.2	10.0	35.2	38.4	9.0	9.8	5.8	155	101	54	73	7.4	26.0
S.	12– 2	23.5	1.014	3.8	0.9	415	98	608	145	66.2	15.6	25.5	6.0	33.3	7.8	145.0	34.0	31.0	7.3	5.3	135	98	37	86	5.6	26.8
R.	2– 4	60.0	1.011	6.7	4.0	544	326	436	292	46.1	27.6	27.8	16.7	19.2	11.5	65.1	39.0	8.5	5.1	5.5	151	97	54	74	7.5	27.2

instrument) methods simultaneously. Each blood pressure and pulse rate figure given in the tables is thus the average of ten readings. No food was taken during the experiment and no water except the 200 cc. at the beginning of each period. A portion of each urine sample was collected with the subject urinating down the side of a large test tube containing paraffin oil, this portion being used for CO_2 and pH determinations. Inorganic phosphates were determined by Briggs' method, chlorides by Austin and Van Slyke's, urea by urease action with direct Nesslerization, ammonia by the permutit method, creatinine by Folin's method (potassium bichromate standard), inorganic sulphate by Fiske's benzidine method, titratable acidity by Folin's technique, bicarbonate as CO_2 by Bishop's modification (1926) of the Van Slyke and Neill constant volume apparatus, and pH colorimetrically, using the Clark and Lubs series. Results of the nine experiments are seen in tables 1 to 9.

Concerning ourselves first only with the question of the mechanism of the water output, we see that in every case the volume output of urine is much greater in the recumbent period than in the corresponding standing period. It apparently makes little or no difference whether the experiment is started with a standing or with a recumbent period. Thus, subject H., on February 6, passed 36 cc. of urine per hour standing and 208 cc. per hour recumbent, when the first period was one of standing; on February 13 he passed 235 cc. per hour recumbent and 54 cc. per hour standing, with the recumbent period first. It will be noted that subjects H. and S. show a greater percentage increase of urine output on recumbency than does subject D.

Richards and Plant (1922) have shown that, under the conditions of their experiments, the rate of urine output is directly proportional to the perfusing pressure, the volume flow remaining constant. We have, in the experiments reported in this paper, approximately the reverse of the conditions obtaining in Richards and Plant's work. That is to say, the mean arterial pressure[2] in every recumbent period is as low as or lower than that in the corresponding standing period (except with subject S.), while the circulation rate is greater. We appreciate, of course, that changes of pressure in the brachial artery are not necessarily a reliable index of changes in glomerular capillary pressure. In Richards and Plant's work, however, the pressure determinations were made at the renal artery and there is no reason to suppose that general blood pressure changes as manifested in the brachial artery are not also occurring at the renal artery. And since Richards and Plant have shown that pressure changes in the renal artery effect corresponding changes in urine flow it is reasonably certain that

[2] This is closely approximated by the sum of the diastolic pressure plus one-third of the pulse pressure.

corresponding changes were taking place in the glomerular capillaries.[3] We believe, therefore, that we are as adequately justified in taking pressure changes in the brachial artery as an index of glomerular capillary changes as were Richards and Plant in so taking the pressure changes in the renal artery. It is also recognized that changes in the general circulation rate do not necessarily signify corresponding changes in the volume flow through the kidneys. With such striking increases in circulation rate as usually occur in the recumbent posture as compared to the standing, however, it is highly probable that the kidneys' blood volume flow participates in the increase, especially since nothing is being done which might be presumed to bring about a selective renal vasoconstriction. If then our premises be granted that we have in the recumbent periods a lower glomerular capillary pressure and a greater kidney blood volume flow than in the standing periods, how is the greater urine output in the recumbent periods to be explained?

The question of the mechanisms involved in the increased urine flow in the recumbent position has been considered by Linossier and Lemoine (1903) and by Erlanger and Hooker (1904). The experimental procedure employed by Linossier and Lemoine was quite different from that in our work. No details are given as to the control of the food and water intake previous to and during the experiments, the periods followed were alternate days of standing and lying instead of two-hour periods, and the statement is made that the subjects were sitting more of the time than standing during the so-called standing period. In spite of these disturbing factors they invariably find a higher urine output in the recumbent position; no blood pressure determinations are reported. They assumed that the lesser urine output on standing is due to a torsion on the renal pedicle which interferes with the blood supply to the kidney. They have, of course, no observations on the circulation rate. Erlanger and Hooker observed a parallelism between magnitude of pulse pressure and urine output, and this parallelism is evident in our data. They present a discussion of the various processes by which a pulsatile effect might influence renal activity. The relation of pulse pressure to renal secretion has since been studied by Hooker (1909), (1910), and by Gesell (1913), who found that in general the amount of urine eliminated varied directly with the magnitude of the pulse pressure. Gesell (1914) has also shown that a

[3] The objection may be raised that when, as in our experiments, a fall of arterial pressure is due to arteriolar relaxation, the pressure in the capillaries should vary inversely as that in the artery. This is true for the circulatory bed in general; in the kidney, however, Richards and Plant have shown that variations in peripheral resistance are effected primarily by variations in the calibre of the efferent arterioles. It is thus seen that conditions in the kidney are peculiar in that glomerular capillary pressure varies in the same sense as does arterial pressure.

pulsatile application of pressure favors the filtration of various colloidal solutions through inanimate membranes.

The increased urine volumes obtained during the recumbent periods in our experiments are clearly due to some other process than an increased mean filtering pressure, this statement, of course, depending upon the apparently justifiable assumption that mean pressure changes in the brachial artery may be taken as an index of mean pressure changes in the glomerular capillaries. The other two factors which are certainly changed in the general circulation and presumably in the glomerular capillaries, are the pulse pressure and volume flow. Which of these factors is more concerned with the increased urine flow it is impossible to state; quite probably both play a part.

Gesell's experiments on changes of pulse pressure alone as a causative factor in changing the rate of urine flow appear convincing, even though it may be difficult to understand the mechanism of such a process. Our conception of the mechanism by which an increased blood volume flow affects the rate of urine flow is greatly clarified by the work of Richards and Schmidt (1924) and of Khanolkar (1922) on the glomerular circulation. The former workers have shown that various influences, as section of the sympathetic nerve supply to the kidney, injection of various salts, glucose, urea, caffeine, and small doses of pituitrin may increase the number of glomerular capillaries exhibiting an active circulation, while stimulation of sympathetic renal nerves, hemorrhage, or large doses of adrenalin or pituitrin decreases the number. It is highly probable that in the recumbent posture the kidney vessels participate in the general relaxation evidenced by the fall of diastolic pressure. The opening up of more glomerular capillaries gives a greater number of filtering units in operation, which would increase the rate of urine flow even without any increase in the filtration rate of an individual glomerulus. While this process in all probability is a factor in the increased rate of urine elimination in the recumbent position, it alone will not explain the changes found in the composition of the urine. For it is evident that doubling the number of active filters without changing the filtration rate of the individual glomeruli should merely double the amount of urine formed, without altering its composition, unless we are to postulate an altered behavior on the part of the tubules toward the glomerular filtrate. We must, then, in order to explain our findings, assume, in addition to an increased number of active filtering units, either an increased filtration rate of the individual units, thereby diminishing the time allotted to the tubular cells for a reabsorptive modification of the filtrate, or a diminution in the reabsorptive function, (or increase in secretory function) of the tubular cells. This point will be considered further in the following part of the paper, where the chemical data are discussed.

TABLE 10

Averages of all experiments on each subject. Circulation rate in liters per minute

SUBJECT	POSTURE	CC. URINE PER HOUR	CO_2, MGM. PER HOUR	NaCl, MGM. PER HOUR	P, MGM. PER HOUR	S, MGM. PER HOUR	UREA N, MGM. PER HOUR	AMMONIA N, MGM. PER HOUR	CREATININE, MGM. PER HOUR	TITRATABLE ACID $\frac{N}{10}$ CC. PER HOUR	pH	SPECIFIC GRAVITY	SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	CIRCULATION RATE
H	S	51	5.5	362	14.9	19.6	262	27.4	56.5	6.1	5.4	1.016	117	89	28	3.6
	R	185	37.6	862	22.1	26.3	457	28.3	60.5	7.5	6.0	1.009	115	72	43	4.8
	Percentage increase in R	263	584.0	138	48.0	34.0	75	3.0	7.0	23.0					54	34.0
D	S	50	2.2	296	14.4	18.3	386	32.8	47.7	9.3	5.0	1.015*	105	71	34	4.8
	R	103	5.8	468	21.8	22.6	488	25.1	52.8	12.6	5.1	1.012	106	62	44	6.7
	Percentage increase in R	106	164.0	58	51.0	23.0	26	−23.0	11.0	36.0					29	40.0
S	S	35	3.8	220	18.4	10.2	166	11.7	41.7	6.2	5.6	1.016	144	101	43	4.5
	R	155	38.0	603	27.8	16.8	319	11.2	46.8	6.7	6.0	1.006	159	101	58	7.1
	Percentage increase in R	343	900.0	174	51.0	65.0	92	−4.0	12.0	8.0					35	58.0
Average increase for all subjects, R		237	549.0	123	50.0	41.0	64	−8.0	10.0	22.0					39	44.0

* Specific gravity not determined on one standing sample of 11.7 cc.

The other aspect of this work deals with the question of whether the tubular cells add anything to the glomerular filtrate. It seemed reasonable on the following basis that the methods of these experiments might throw some light on this question. If the increased urine flow in the recumbent position is due to an increased glomerular filtration, one might expect the output of those urinary constituents which are eliminated solely by a filtration process to be increased to a greater extent than would the output of those constituents which are eliminated in part by filtration and in part by tubular secretion. If, then, the urinary constituents can be divided into two groups, one showing a higher percentage increase of output in the recumbent position, as compared to standing, than does the other, the presumption is that the group showing the lesser augmentation of output in the recumbent position is in part eliminated by secretion. The following table is made up of the averages of the results obtained on each subject. The figures on subject H. represent the average of eight standing and of eight recumbent periods, on subject D. of six standing and six recumbent, and on subject S. of four standing and four recumbent.

It is apparent that the percentage increase in output of bicarbonate (549 per cent), water (237 per cent) and chloride (123 per cent) is far greater than that of any other urinary constituent. Phosphate (50 per cent) sulphate (41 per cent) and urea (64 per cent) are moderately increased, while ammonia (−8 per cent) and creatinine (10 per cent) are practically (in the average) unchanged. In regard to ammonia the tables of the individual experiments, 1 to 9, show that the ammonia output per hour is sometimes greater in the standing periods, sometimes in the recumbent. This is to be expected as a result of the organism's varying need for acid neutralization. The important point is that ammonia, unlike bicarbonate, water and chloride, bears no relation at all to the presumptive rate of glomerular filtration. The rate of creatinine elimination is very little changed by the change of posture; it is usually but not invariably somewhat increased in the periods of recumbency. The work of Nash and Benedict (1921), and of Behre and Benedict (1922) makes it practically certain that ammonia and creatinine are largely secreted by the tubules, and it is thus to be expected that there should be no consistent marked change in their output on changes of glomerular filtration rate. We have believed for some time that phosphates, sulphate and urea are in part filtered through the glomeruli, and in part secreted by the tubules. If this view is correct one should expect some increase in the output of these bodies when glomerular filtration is increased, but not so great an increase as in the output of bodies which are eliminated solely by filtration. Our finding that bicarbonate and chloride are most increased, ammonia and creatinine practically unchanged, and urea, phosphate and sulphate increased to a much less extent than bicarbonate and chloride, is in accord with the view that bicar-

bonate and chloride are eliminated solely by filtration, ammonia and creatinine almost solely by secretion, and urea, phosphate and sulphate by a combination of these processes.

We further believe that the data afford information as to the relative importance of the filtration and secretory processes in the elimination of urea, of phosphate and of sulphate, respectively. Since the urea output is increased in the recumbent periods more than is the output of phosphate and sulphate (average 64 per cent increase in urea, 50 per cent in phosphate and 41 per cent in sulphate) it is probable that the ratio of urea filtered to urea secreted is higher than is the ratio of phosphate filtered to phosphate secreted, or of sulphate filtered to sulphate secreted. Another reason for this belief is that practically always the urea output is considerably greater in the recumbent than in the standing periods, which we interpret as evidence that the filtration process is playing a relatively important rôle in urea elimination, since this process is almost certainly the one most affected by postural changes. The phosphate and sulphate, on the other hand, while they usually show a greater output in the recumbent period, may show a considerably greater output in the standing period. We interpret the occasional markedly greater output of phosphate or sulphate in the standing period as being due to an increased secretion of phosphate or sulphate in this period, which more than compensates for the smaller amount filtered.

We would point out here the difficulties of interpreting these data on the view that all the urinary constituents (except ammonia and creatinine) are eliminated solely by filtration. On this view one would assume that in the recumbent periods the filtration rate is increased to such an extent that a larger proportion of the water, bicarbonate and chloride escapes reabsorption. But one must further assume that in the recumbent periods the reabsorption of urea, phosphate and sulphate is much more complete than is that of bicarbonate and chloride, since the former fail to show anything like the same increase in output in the recumbent periods as do bicarbonate and chloride. This assumption appears wholly unreasonable; it would mean that the organism shows a greater tendency to conserve the waste products of protein metabolism than it shows for the useful bicarbonate and chloride; it would be not merely a denial of Cushny's original no-threshold body idea but a statement that the opposite is true. The only other assumption on which the filtration-reabsorption advocates can explain the present data is that in the recumbent position glomerular filtration is unchanged or increased only to the extent that the output of urea, phosphate and sulphate is increased; the greatly increased output of water, bicarbonate and chloride must then be explained on the assumption that in recumbency the capacity of the tubules to reabsorb these substances is greatly diminished. We know of no a priori reason or experi-

mental evidence for either of these assumptions; surely neither of them seems as reasonable to us as an explanation based on variations in rate of glomerular filtration with corresponding changes in time allowed for tubular modification of the glomerular filtrate,[4] for which there is at least abundant indirect evidence; e.g., the well known tendency of the composition of the urine to approach that of the plasma, at least in respect to most of its constituents, as diuresis becomes more intense, and the more direct evidence in the observations of Richards and Plant, that with a rise of perfus-

[4] Marshall and Crane (1924) have objected to two assumptions made by one of us in an earlier publication (White, 1922), which were the basis of an argument in favor of a tubular secretion of urea, sulphate and phosphate (and of sugar in the phlorhizinized dog). These assumptions were, to quote Marshall and Crane's statement of White's view, "that if the rate of urine flow from the ureter is increased in one period compared to another of any given experient, the rate of glomerular filtration is increased or at least not reduced in the period of greater urine flow; and that when the rate of urine flow is greater in one period than in another the reabsorption of any body must be less in the case of the period of greater urine flow." They further state, "We do not believe that either of these assumptions is necessarily true; and as no evidence for their validity has been presented, arguments based upon them do not prove the secretion of urea (or of the other substances considered)." Marshall and Crane's skepticism in regard to the first assumption apparently means that they regard changes in rate of urine flow as due primarily to changes in the rate at which the tubules reabsorb water, rather than to changes in rate of glomerular filtration. This view, as applied to these experiments, would necessitate the assumption that the mere act of lying down invariably greatly diminishes this reabsorptive capacity of the tubules. We cannot see how this assumption is reasonable; it is, on the other hand, in our opinion, clear that the circulatory changes induced on taking the recumbent position should increase the rate of a filtration process; even though absolute proof for this cannot be advanced it at least seems more reasonable than the alternative assumption that a changing water reabsorptive capacity of the tubules is the sole mechanism by which wide variations in rate of urine flow can be effected. By "changing reabsorptive capacity of the tubules" is meant, of course, a change in the ability of the tubules to reabsorb due to changes in the activity of the cells, as opposed to a change due merely to a diminished time available for reabsorption. The supporters of the filtration-reabsorption theory may say that they have never maintained that variations in water reabsorptive capacity of the tubules are the *sole* mechanism regulating wide variations in rate of urine flow, but they are forced to this assumption, whether or no. For, if all the phosphate, sulphate and urea are filtered, they cannot admit that any greater percentage changes take place in rate of glomerular filtration than in rate of bladder output of phosphate, sulphate and urea, unless they make the further wholly indefensible assumption that in periods of rapid glomerular filtration the tubules *are reabsorbing the waste products*, phosphate, sulphate and urea, much more efficiently than in the periods of slow glomerular filtration.

Even though it be accepted that the changes in rate of urine flow in the present experiments are due primarily to changes in rate of glomerular filtration, it does not, to be sure, necessarily follow that the changes in rate of urine flow in the experiments criticized by Marshall and Crane were due to the same process; evidence that they were is presented in the following paper.

ing pressure the urine flow becomes more rapid, and "the fluid issuing from the ureter comes to take on more and more the character of a blood filtrate." We regard the inadequacy of the explanations of the data based on the filtration-reabsorption hypothesis as evidence in favor of a secretory process.

Another question of interest is whether the increased urine output in the recumbent periods is due to a greater number of active filtering units, or to a more rapid filtration rate of the individual units, or to a combination of these processes. The other conceivable possibilities are, of course, an increased tubular secretion or diminished reabsorption of water or a combination of these. To consider the latter possibilities first, we do not believe that the data give any indication of a consistently increased secretion of any of the urinary *solids* in recumbency; such increases as consistently occur are, in our opinion, more reasonably explained on the basis of an increased filtration.[5] It seems to us, then, gratuitous to suppose that there is a consistent increase in tubular secretion of *water* in recumbency. The other possibility, a diminished reabsorptive activity, has been discussed in the preceding paragraph. There is surely no a priori reason for assuming a diminished water reabsorptive capacity of the tubules in the recumbent posture, and since circulatory changes are occurring which almost certainly affect the filtration process it appears to us justifiable to make the assumption that the changes in urine output are due to one or the other, or both of the filtration changes mentioned in the first sentence of this paragraph. The following arguments stand or fall with the truth or falsity of this assumption. The evidence for it has been presented; to us it seems more reasonable than the alternative assumption of a changing reabsorptive capacity of the tubules. The two assumptions are not mutually exclusive; the question reduces itself to which factor predominates in determining the rate of urine output under the conditions of these experiments.

[5] This does not mean that there is never an increased tubular secretory activity in recumbency; it may be increased or decreased, depending on the state of the organism; there are probably other factors (hormonal ?) than the plasma concentration of a constituent which determine the activity with which the tubular cells will eliminate that constituent. The point we are trying to make is, that with the view that only a relatively small part of the phosphate, sulphate and urea is filtered, the remainder being secreted, great variations in amount of glomerular filtrate are not inconsistent with the finding of relatively slight variations in their output. While with the view that all the phosphate, sulphate and urea are filtered, one is forced to the assumption that the variations in amount of glomerular filtration are no greater than the variations in the amounts of phosphate, sulphate and urea reaching the bladder, unless one is willing to make the further assumption, which seems to us wholly unreasonable, that during a period of greatly increased glomerular filtration, there is also a great increase in the capacity of the tubules to reabsorb phosphate, sulphate and urea.

As has already been mentioned, it is highly probable that the factor of an increased number of active filtering units is operating in the increased recumbent flow, but it is certain that there is also an increased rate of filtration by the individual glomeruli. This is evidenced by the marked changes in the percentage composition of the urine. The constant finding of an increased concentration of bicarbonate and water in the periods (recumbent) of greater urine flow means to us that a more rapid filtration is occurring in the individual glomeruli. We have already stated our reasons for the belief that during the periods of greater urine flow the mean filtering glomerular pressure (except in subject S.) is lower than in the standing periods, while the pulse pressure (including the case of subject S.) is higher. We believe, then, that these experiments afford further support to the view advanced by Erlanger and Hooker, by Hooker, and by Gesell that a pulsatile application of pressure favors a filtration process. It appears that filtration may actually proceed more rapidly with a slightly lower mean filtering pressure and a higher pulse pressure, than with a slightly higher mean pressure and a lower pulse pressure. The fact that the percentage increase in output of water, bicarbonate and chloride in the recumbent periods as compared with standing is greater with subject S., whose mean pressure as well as pulse pressure was increased in the recumbent periods, is consistent with the view that the mean filtering pressure as well as the pulse pressure is a causal factor in the rate of glomerular filtration.

We have not discussed the figures on titratable acidity and urine pH; their values depend upon a complexity of factors which at the present stage would render a discussion, at least one by the authors, of their bearing on the question of the renal mechanism only confusing.

SUMMARY

Data are presented showing the influence of posture on the urinary output of water, bicarbonate, chloride, urea, phosphate, sulphate, ammonia, creatinine, titratable acid, on urine pH, on blood pressure, pulse rate and circulation rate in nine experiments on three subjects.

Arguments are presented leading to the following conclusions: that urea, sulphate and phosphate are in part filtered through the glomeruli and in part secreted by the tubules; that the rate of filtration across a glomerular membrane is determined not only by the mean glomerular capillary pressure but by the extent to which the application of pressure is pulsatile; that the greater urine flow in the recumbent position is due in part to an increase in the number of glomerular capillaries exhibiting an active circulation, and in part to an increased rate of filtration by individual glomeruli.

The validity of the arguments leading to these conclusions depends upon certain assumptions which we believe to be more reasonable than alterna-

tive assumptions which, as we see it, must be accepted if ours are rejected. Arguments are presented for our belief that the assumptions taken as the premises of this paper are more reasonable than the alternative ones.

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FURTHER EVIDENCE ON THE RELATION OF THE FILTRATION PROCESS TO DIURESIS

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In a series of papers by White (1923) the conclusion was reached that phosphate, sulphate and urea (and sugar in the phlorhizinized dog) are in part secreted by the tubules. An assumption on which the arguments leading to this conclusion were based was that, under the conditions of the experiments reported, the rate of glomerular filtration was at least as high in the periods of rapid urine flow as in those of slow urine flow. Marshall and Crane (1924) have made the criticism that since no evidence was presented for the validity of this assumption, "arguments depending on it do not prove the secretion of urea (or of the other substances considered)." Their criticism apparently means that they consider changes in rate of urine flow as due primarily to changes in the capacity of the tubules to reabsorb water, rather than to changes in rate of glomerular filtration. We believe that the experiments reported in this paper afford evidence for the validity of the assumption that during a diuresis produced by the intravenous injection of sodium chloride solution in a dog, the rate of glomerular filtration is higher than during a period of slower urine flow. This does not, of course, exclude the possibility of changes in the reabsorptive capacity of the tubules; we have never maintained that no such changes can occur, and the possibility of their occurrence does not prejudice the validity of the assumption on which our earlier arguments were based.

The dogs used in the experiments reported here were given as nearly as possible the same treatment as that given the dogs used in the earlier work. They were given a grain of morphine subcutaneously per ten kilos body weight, anesthetized with ether, a tracheal cannula was inserted and an injection of sodium chloride solution made into the femoral vein. Urine was collected from a catheter in the bladder, the free end of the catheter dipping under paraffin oil. The collecting bottles were weighed before and after the collections; the figures expressing output of urine are thus grams of urine per hour rather than cubic centimeters per hour. All collection periods were one-half hour. The mean blood pressure was recorded by a

mercury manometer from a femoral artery, blood samples were drawn from the other. Very little ether was required after the cannulas were inserted. Protocols and data of two experiments follow.

In the preceding paper (White, Rosen, Fischer and Wood, 1926) it was pointed out that the increase in output of water, bicarbonate and chloride on changing from the standing to the recumbent posture is almost cer-

TABLE 1

Experiment 1. Male dog, body weight 18.7 kilos. Thirty cubic centimeters per kilo per hour of 0.7 per cent NaCl solution intravenous injection started at 11:10 (15 minutes before beginning first urine collection) and continued until 12:25, i.e., the end of the second collection period. At 12:25, 40 cc. per kilo per hour of 1.8 per cent NaCl solution was started and continued until 2:05, the end of the last collection period. In all cases the salt solution was at body temperature and was injected intermittently rather than continuously. One-twelfth of the hourly amount was injected every five minutes, thus avoiding the necessity of constantly regulating the pressure to keep rate of inflow constant. The fourth urine sample was lost. Blood samples were drawn in the middle of each urine collection period.

TIME	MEAN BLOOD PRESSURE	BODY TEMPERATURE	URINE PER HOUR	CO_2 PER 100 CC.	CO_2 PER HOUR	NaCl PER 100 CC.	NaCl PER HOUR	pH URINE	PLASMA, NaCl PER 100 CC.
			grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*		*mgm.*
10:55	125								
11:10	109	38.1							
11:30	120								
11:40	114	38.0	48.4	7.8	3.8	835	404	6.2	683
11:55	110								
12:10	121	38.0							
12:20	122		104	8.9	9.3	651	677	6.2	685
12:30	114								
12:45	117		235	10.8	26	752	1767	6.3	720
1:00	130								
1:10	135	38.0	Urine sample lost						720
1:20	134								
1:40	134		350	12.4	43	755	2642	6.45	759

tainly due mainly to an increased filtration, the only alternative to this view being the assumption that the mere act of lying down greatly diminishes the capacity of the tubules to reabsorb these substances. Since change of posture cannot conceivably alter the composition of the blood or create a surplus of water, bicarbonate or chloride in the organism, and since the probable increase in blood volume flow to the kidneys in recumbency should, if anything, render the tubular cells capable of a more active

reabsorptive process, it is clear that this assumption is quite untenable and the proof seems convincing that the postural changes in output of water, bicarbonate and chloride are mainly due to changes in the filtration process. As was mentioned in the preceding paper, however, it does not necessarily follow that the changes in urinary output produced by intravenous injection of salt solution in a dog are also due to changes in the

TABLE 2

Experiment 2. Male dog, body weight 15 kilos. Twenty cubic centimeters per kilo per hour of 1.8 per cent solution of NaCl intravenously started at 10:45 (15 minutes before beginning the first urine collection) and continued until 11:30. Thirty cubic centimeters per kilo per hour of 1.8 per cent NaCl solution started at 11:30 and continued until close of experiment.

TIME	MEAN BLOOD PRESSURE	ROOM TEMPERATURE	BODY TEMPERATURE	URINE PER HOUR	CO_2 PER 100 CC.	CO_2 PER HOUR	NaCl PER 100 CC.	NaCl PER HOUR	pH URINE	PLASMA, NaCl PER 100 CC.	PLASMA PER CENT PROTEIN
				grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*		*mgm.*	
10:45	140		37.0								
10:50	144	25.0	37.0								
11:00	140	25.0	36.8								
11:10	152	25.0	37.0	60	48	29	2077	997	7.2	730	5.05
11:20	148	25.0	37.0								
11:30	138	25.1	37.0								
11:40	142	25.0	37.0								
11:50	148	25.0	37.0	133.6	50	67	1490	1990	7.2	773	4.7
12:00	136	25.0	37.0								
12:10	138	24.9	37.0								
12:20	132	25.3	37.0	198	57	113	1185	2346	7.25	805	4.41
12:30	132	25.3	37.0								
12:40	138	25.2	37.1								
12:50	134			220.2	58	127	1040	2290	7.35	833	4.06
1:00	125	25.6	37.0								
1:10	122										
1:20	118	25.8	37.0	268	54	145	837.5	2244	7.35	868	3.94
1:30	117	25.3	37.1								

filtration process. Critics of the assumption that the rate of glomerular filtration is at least as high in the periods of rapid urine flow as in those of slower urine flow may admit that the assumption is valid in the case of variations in urine output brought about by postural changes in man and still not admit that it holds for a diuresis produced in a dog by the intravenous injection of salt solution. For while in the experiments reported in the preceding paper there is no reason to assume a surplus in the organ-

ism of water, bicarbonate or chloride in the recumbent position, there is ample justification for the view that an intravenous injection of salt solution is creating in the dog a surplus of water and chloride. They would have, therefore, a reasonable basis for the attitude that the increased output of water and chloride during the periods of diuresis might just as satisfactorily be explained on the view that the tubules were failing to reabsorb these bodies as on the view that a greater amount was being filtered. We believe, however, that the bicarbonate figures in the experiments reported here answer the question beyond a reasonable doubt in favor of an increased filtration process as being the main factor responsible for a diuresis produced by intravenous injection of salt solution. For it is evident from the data that the output of bicarbonate is increased, as the injection proceeds, as much as or more than that of water and chloride, although there is no surplus of bicarbonate being created, no alkalosis or change of any kind which might be presumed to diminish the avidity with which the tubular cells should reabsorb bicarbonate. The only reasonable explanation seems to us that of an increased filtration, with the result that a lower percentage of the total amount filtered is reabsorbed.

If this view of an increased filtration during diuresis is accepted, it is impossible,[1] as has been pointed out in the preceding paper, to explain on the filtration-reabsorption basis the findings (which, so far as we can learn from our own experience and from the literature, always obtain during a diuresis) of a far greater increase in the output of water, bicarbonate and chloride than in the output of urea, phosphate and sulphate. The findings are clearly explicable, however, on the view that a considerable proportion of the urea, phosphate and sulphate is eliminated by tubular secretion; a large increase in filtrate would then cause only a relatively small percentage increase in the total amount of these bodies eliminated.

We might point out here that the second assumption made in White's earlier paper, while it is probably true, is not really essential to the development of the argument in favor of tubular secretion.[2] The admission of

[1] Unless one makes the untenable assumption that during a period of rapid filtration the tubules are able to reabsorb urea, phosphate and sulphate very much more efficiently than water, bicarbonate and chloride.

[2] Marshall and Crane's statement of the second assumption, "that when the rate of urine flow is greater in one period than in another the reabsorption of any body must be less in the case of the period of greater urine flow," is really stronger than our original statement. Substitution of the words, "will not be greater" for "must be less," in the above statement expresses the assumption as stated by White. To avoid possible confusion, we would call attention here to an error in Marshall and Crane's paper, namely, the use of the word "lesser" for the word "greater" in the tenth line from the bottom on page 483. Their note calling attention to this error, which appeared in the next number of the Journal, may have escaped the notice of some readers.

an increased glomerular filtration during diuresis is in itself sufficient to establish the point with practical certainty.

SUMMARY

Experimental evidence is presented that a diuresis produced by intravenous injection of sodium chloride solution in an anesthetized dog is accompanied by a greatly increased output of bicarbonate.

The fact that under the experimental conditions obtaining in the work reported in an earlier paper a diuresis is accompanied by a greatly increased output of bicarbonate is interpreted as evidence for the validity of an earlier assumption that during diuresis the rate of glomerular filtration is at least as rapid as during a period of slower urine flow.

The unreasonableness of the assumptions which must be made in order to explain the elimination of the urinary constituents (ammonia and creatinine excepted) solely on the basis of filtration, is pointed out.

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THE REGULATION OF RESPIRATION

III. A Continuous Method of Recording Changes in Acidity Applied to the Circulating Blood and Other Body Fluids[1]

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The importance of a constant hydrogen ion concentration of the arterial blood to normal tissue function has been greatly stressed within the past decade. The apparent dependency of tissue acidity on blood acidity coupled with the constancy of the arterial hydrogen ion concentration in the normal individual seemed to justify this emphasis. This constancy of hydrogen ion concentration of the arterial blood was attributed to the extreme sensitivity of the respiratory center to the free hydrogen ion of the blood. In fact this view became so firmly established that the absence of a demonstrable increased acidity of the arterial blood during hyperpnea was considered indicative of the supersensitivity of the respiratory center to the present physico-chemical methods of detecting the hydrogen ion. It is, therefore, not surprising that the technique for the determination of the hydrogen ion concentration of the blood has advanced so rapidly within the past few years. It seems now, however, that the problem of respiratory control must be considered from a somewhat different angle. Direct experiments (1) and the review of extensive literature (2) indicate a gross insensitivity of the respiratory mechanism to the free hydrogen ion of the arterial blood. Indeed, the relatively greater frequency of the inverse relation of pulmonary ventilation to the hydrogen ion concentration of the blood indicates that a causal relationship is wanting. Hence a detailed elucidation of the relation of pulmonary ventilation to the hydrogen ion concentration of the blood seemed of sufficient interest to warrant an effort to develop a continuous method of recording changes in the hydrogen ion concentration of the circulating blood. The advantages of a continuous method in determining the time relation of acidity changes to

[1] Proceedings of the Society for Experimental Biology and Medicine, 1925, xxii, 298–300. This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

pulmonary ventilation, oxygen consumption, blood pressure, heart rate, etc.; the possibility of recording synchronously changes in acidity of the arterial and venous blood, cerebro-spinal fluid (3), urine (4), and other body fluids; the large amount of data obtainable as compared with the discontinuous method of sampling—offer invaluable opportunities for advancing the subjects of respiratory control and acid-base equilibrium.

METHODS. The experiments of Roaf (5) on the time relation of acid production in muscle during muscle contraction suggested the use of the manganese dioxide electrode studied by Tower (6) and Smith (7). The electrode has two advantages which particularly recommend its use. As it functions independently of the pressures of hydrogen and oxygen it excludes the necessity of saturation of the blood with hydrogen, and permits the recording of changes in blood acidity in the presence of synchronous changes in oxygen pressure of the blood. On the other hand, it is stated that the absolute values obtained with the electrode are unreliable. This may appear to be a serious objection, but we believe that the short observations of the acute experiments showing rapid changes in blood acidity and pulmonary ventilation may, for a time at least, lead to more rapid progress in the study of respiratory control than the accurate determination of the hydrogen ion concentration of the blood in long-established conditions of equilibrium. Provided the electrode gives significant information on the magnitude and direction of changes in acidity it should prove to be a valuable tool.

The development of the use of the manganese dioxide electrode resolved itself into several distinct problems: the mechanical registration of changes in acidity, the preparation of a suitable electrode, the establishment of the validity of data yielded by the electrode, and the application of the method to the circulating blood and other body fluids of the living animal. The difficulties were varied and numerous, some are still to be overcome. Inasmuch as the behavior of the manganese dioxide electrode, especially in complex fluids, is imperfectly understood many of our difficulties were solved by purely empirical methods of trial and error; but by gradual elimination we have adopted methods which yield significant data.

In vitro experiments. The manganese dioxide electrodes were placed in the stream of fluids studied. The fluids were kept at a constant rate of flow by means of a mechanically operated syringe of 100 cc. capacity and a three-way stop cock, which allowed an alternate change of fluids of different pH values. The potentiometer circuit was closed with a saturated potassium chloride calomel electrode, as shown in figures 1 and 2. By using four potentiometers (the type K Leeds & Northrup potentiometer proved a very satisfactory instrument) it was possible to obtain four synchronous records and thus test for the reproducibility of behavior of electrodes under

similar conditions. Permanent records of changes in acidity were made on smoked paper with the mechanical method employed by one of us in registering the electrical deflections of the submaxillary gland (8)—the method differing in that the galvanometer was maintained at zero and the compensating E.M.F. recorded by vertical writing points attached by thread to spindles mounted on the potentiometer drum. The spindles were of 1.655 cm. diameter, which gave a vertical deflection of 52.0 mm. per 0.01 volt change in E.M.F. The smoked paper accommodated changes in E.M.F. of approximately 0.05 volt. The initial level of the record was conveniently placed at any height on the drum by a rachet device on the spindle, one complete turn representing 0.01 volt. Not infrequently the

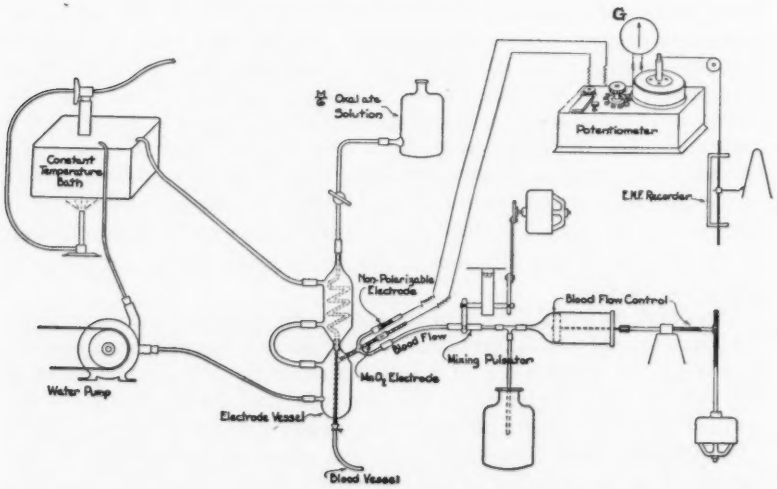

Fig. 1. In vivo method for recording changes in acidity of the blood with the use of sodium oxalate as an anticoagulant.

initial position of the drum preceding an observation may be at its upper limit (lowest E.M.F.) or at its lowest position (highest E.M.F.). If the change in pH is in the alkaline direction the first position is awkward for registering the change, and if in the acid direction the second position is equally inconvenient. Either position requires an adjustment of the coarse resistance followed by a complete shift of ten revolutions of the sliding contact. To avoid this inconvenience and loss of continuity of the record a 5 ohm resistance was placed in parallel with one of the 5 ohm resistance coils of the coarse adjustment and the working current readjusted. This

brings the sliding contact to the mid position which is favorable for registering changes in acidity in either direction.

The ideal electrode should possess the properties of sensitivity, rapidity of response, sturdiness and reproducibility. It soon became apparent that these conditions were hard to meet. The thinly plated electrode though sensitive and rapid in response was quick to dissolve and, therefore, unsatisfactory for prolonged experiments. The thickly coated electrode was found to possess durability but was sluggish in response and less sensitive to changes in acidity. A compromise was reached between durability on the one hand and sensitivity and quickness of response on the other by adjusting the duration of plating. Reproducibility we accomplished by following a standard routine of preparation. The following procedure was adopted.

A piece of no. 24 platinum wire about 6 to 8 mm. long was sealed into the end of a glass tube 2 mm. inside diameter, 3 mm. outside diameter, and 7 cm. long. The protruding end, about 1 mm. in length, was rounded on a fine stone (to avoid point effects) then heavily plated with platinum black and heated to red heat in the alcohol flame. This electrode connected with the positive pole of a 6 volt battery was plated for one and one-half minutes in acidified (H_2SO_4) 0.4 N solution of manganese sulphate with 650 ohms resistance in the external circuit. The negative electrode of similar construction was placed 2 cm. from the positive electrode. A 2 N manganese sulphate solution which served as a stock solution was freshly prepared every seven days. To avoid the annoyance of chance poisoning of the platinum, new platinum was used for each electrode. We are not certain that these precautions are necessary, but scrupulous cleanliness and the use of fresh materials have materially decreased irregularity in behavior of the electrodes.

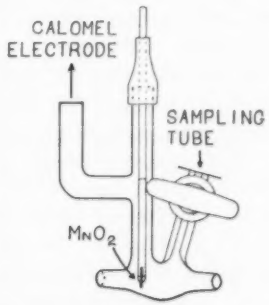

Fig. 2. Combination electrode and sampling cannula for recording changes in acidity of the circulating blood in the heparinized dog.

Since the freshly prepared electrodes show an alkaline drift—rapid at first but gradually diminishing in rate—they were equilibrated in the fluids tested. It appears that complete equilibration was seldom obtained. In phosphate buffer mixtures the drift was rapid and mostly over within fifteen to thirty minutes. In egg albumen, blood plasma, and whole blood a slow drift persisted for several hours. The electrodes were, therefore, equilibrated for three or four hours before using. It seems probable that the more prolonged drift in protein solution is associated with the formation

of a protein film on the electrode.[2] Drift obviously is an important factor in reproducibility of absolute E.M.F. values for a given pH.

Tower reports a high grade of reproducibility of electrodes with variations in absolute E.M.F. values amounting only to 0.01–0.02 pH. With the use of the formula $\pi = \frac{RT}{nF} \ln \frac{C_{Mn} C_{H'}^4}{C_{Mn'} C_H^4}$ he determined the pH values. Smith, on the other hand, was unsuccessful in obtaining the same reproducibility of results—particularly with a group of acids designated "inconstant;" and with the "constant" group of acids he found that $\pi = \frac{RT}{nF} \ln \frac{C_{Mn} C_{H'}^{3.56}}{C_{Mn'} C_H^{3.56}}$ agreed better with his observations.

Inasmuch as π is determined not only by the concentration of the H ions but by the Mn ions as well, and as we made no effort to control the concentration of Mn ions, we could hardly expect to reproduce the absolute E.M.F. values of Tower and Smith even though we had confined our experiments to the simple acid solutions which they employed. Nevertheless some of the results which we have obtained may be of interest in indicating the behavior of the electrode—particularly in complex organic solutions. The data in table 1 are collected from experiments on blood plasma, egg albumen, and phosphate mixtures. The third column gives the pH values of the fluids determined with either the hydrogen or the quinhydrone electrode (9). The fourth column gives the corresponding E.M.F. values observed with the manganese dioxide electrode. Column five gives the corrected E.M.F. values for pH 7.4. Column six gives the drift in volts per hour.

For a pH value 7.4 the table shows absolute E.M.F. values ranging from 0.2547 to 0.4809 volts—a difference in terms of pH of approximately 2.3. The explanation of this enormous difference is uncertain but it probably is not due to drift. Inasmuch as the electrodes were uniformly treated (two hours' equilibration in a stationary vessel followed by one and one-half

[2] The existence of such a film may be demonstrated by the electrolytic evolution of hydrogen or oxygen on the electrode, when the gas bubbles cause the separation of a gray film from the surface of the electrode. This is further demonstrated in an experiment with two egg albumen solutions of the same pH but different NaCl concentrations. The direction of E.M.F. change of the MnO_2 electrode on changing from one of these solutions to the other, may be predicted from the difference in NaCl concentration. The effect, however, is not large and is temporary. The MnO_2 electrode soon comes back to its original E.M.F. This effect can be obtained only with fairly large variation in salt concentration and is probably of no importance in the application of the electrode to body fluids. It, however, probably accounts for the irregularities in behavior of the MnO_2 electrode associated with chagees in arterial blood pressure—irregularities that were observed when we used the electrode in the blood-stream, employing sodium oxalate an an anticoagulant.

TABLE 1

Data from in vitro experiments showing E.M.F. values for pH 7.4

EXPERIMENT	FLUID USED	OBSERVED pH WITH (H) HYDROGEN ELECTRODE OR (QH) QUINHYDRONE ELECTRODE	OBSERVED E.M.F. WITH MnO_2 ELECTRODE	MnO_2 E.M.F. VALUES CORRECTED FOR pH OF 7.4*	DRIFT IN VOLTS PER HOUR EQUILIBRATION
12	Plasma	H 7.534	0.2557	0.2547	
13	Plasma	QH 7.605	0.2503	0.2734	0.0046
			0.2478	0.2709	0.0037
			0.2506	0.2737	0.0042
			0.2497	0.2728	0.0053
14	Plasma	QH 7.574	0.2555	0.2758	0.0029
			0.2548	0.2721	0.0037
			0.2513	0.2686	0.0022
			0.2476	0.2649	0.0027
15	Plasma	QH 7.466	0.2590	0.2680	0.0004
			0.2588	0.2669	0.0003
			0.2695	0.2776	0 0006
			0.2577	0.2658	0.0009
16	Plasma	QH 7.456	0 2907	0.2970	0.0015 †
			0.2728	0.2791	0.0013
			0.2930	0.2993	0.0016
			0.2803	0.2866	0.0028
11	Egg albumen	QH 6.736	0.4708	0.4159	0.0132
			0.4701	0.4152	0.0133
			0.4563	0.4014	0.0118
			0.4648	0.4099	0.0164
3	Phosphate	QH 7.217	0.4585	0.4355	0.0005
			0.4584	0.4354	0.0010
			0.4647	0.4417	0.0012
			0.3758	0.3528	0.0206‡
4	Phosphate	QH 7.237	0.4929	0.4798	0.0072
			0.4879	0.4748	0.0034
			0.4990	0.4859	0.0030
			0.4940	0.4809	0.0012

* The data for computing E.M.F. values for pH 7.4 are found in the last column of table 2.

† Acid drifts. Probably due to increasing acidity from bacterial decomposition.

‡ Precipitation about electrode while plating.

TABLE 2

Data from in vitro experiments showing E.M.F. changes of MnO_2 electrodes with changes in hydrogen ion concentration

EXPERIMENT	FLUID USED	OBSERVED pH WITH (H) HYDROGEN ELECTRODE OR (QH) QUINHYDRONE ELECTRODE	pH CHANGE	NUMBER OF MnO_2 ELECTRODES	E.M.F. CHANGE OF MnO_2 PER 0.1 pH MV.
1	Phosphate	H 7.2 –7.4	0.20	1	8.55 7.84
2	Phosphate	H 7.321–7.492	0.171	3	9.10 8.54 8.55
3	Phosphate	QH 6.948–7.217	0.269	4	8.21 8.12 8.05 8.47
4	Phosphate	QH 6.969–7.237	0.268	4	8.12 8.17 8.23 8.26
5	Phosphate	QH 6.858–7.237	0.379	4	7.97 8.00 8.07 8.07
6	Egg albumin	H 7.546–8.355	0.809	1	6.19
7	Egg albumin	H 6.98 –7.123	0.143	2	10.05 8.57
8	Egg albumin	H 6.767–6.869	0.102	1	9.02
9	Egg albumin	H 6.764–7.124	0.360	1	9.93
10	Egg albumin	H 7.739–7.881	0.142	1	7.38
11	Egg albumin	QH 6.428–6.736	0.318	4	8.33 7.69 9.53 7.58
12	Plasma	H 7.743–7.534	0.209	2	8.00 8.45
13	Plasma	QH 7.605–7.782	0.177	4	11.08 11.18 11.31 11.42

TABLE 2—*Concluded*

EXPERIMENT	FLUID USED	OBSERVED pH WITH (H) HYDROGEN ELECTRODE OR (QH) QUINHYDRONE ELECTRODE	pH CHANGE	NUMBER OF MnO_2 ELECTRODES	E.M.F. CHANGE OF MnO_2 PER 0.1 pH MV.
14	Plasma	QH 7.574–7.776	0.202	4	9.59 9.74 10.20 10.24
15	Plasma	QH 7.194–7.466	0.272	4	12.13 12.17 12.44 12.62
16	Plasma	QH 6.716–7.456	0.740	4	10.92 10.97 11.57 11.74

TABLE 3

Summary of results of in vitro calibration experiments

pH DETERMINED BY	IN PHOSPHATE			IN EGG ALBUMIN			IN PLASMA		
	E.M.F. change of MnO_2 electrode per 0.1 pH mv.	Number of electrodes	Number of experiments	E.M.F. change of MnO_2 electrode per 0.1 pH mv.	Number of electrodes	Number of experiments	E.M.F. change of MnO_2 electrode per 0.1 pH mv.	Number of electrodes	Number of experiments
H_2 electrode. ...	8.60 (8.20–9.10)	4	2	8.52 (6.19–10.05)	6	5	8.22 (8.00–8.45)	2	1
Quinhydrone electrode.....	8.12 (7.97–8.47)	12	3	8.28 (7.69–9.53)	4	1	11.21 (9.59–12.62)	16	4
Average.	8.23			8.33			10.87		

hours in a rocking vessel), and inasmuch as the drift following such equilibration was at most 0.005 volt per hour—the main difference in E.M.F. values must be due to some other factor. This factor may be a specific reducing effect of the fluid though this is a point which we have not studied in detail and, therefore, do not care to press. It is suggested, however, as an explanation of the difference in E.M.F values found in the blood plasmas of different animals.

The maximum difference in blood plasmas in our experiments amounted to 0.0446 volt or approximately 0.45 pH. Such differences in biological

work constitute a gross error, but as our main interest is to study changes in pH this variability in absolute values loses significance and especially so if the base line for any particular observation is set by a single determination with methods yielding absolute values. The question of greater concern is the reproducibility of the behavior of electrodes under similar conditions. This reproducibility of behavior is best demonstrated by running several freshly prepared and equilibrated electrodes at the same time in the same fluid. For reproducibility of absolute values under such conditions, see table 1. Note experiment 13, in which absolute values obtained with four electrodes checked within 0.03 pH. Note also the uniformity of drift of approximately 0.04 pH per hour for each electrode. Such uniformity of behavior of electrodes is highly desirable particularly for quantitative comparison of synchronous changes in acidity in the arterial and venous blood, the cerebro-spinal and other body fluids.

For reproducibility of behavior to changes in pH in similar and dissimilar fluids, see tables 2 and 3 and figure 3. Figure 3 shows simultaneous records obtained with four electrodes in the stream of plasmas with alternating pH values of 7.605 and 7.782. The curves are mutually superimpossible despite the alkaline drift. Compared with other methods of determining acidity the records obtained with the manganese dioxide electrode show a degree of uniformity which one could hardly hope to duplicate with any discontinuous method. Figure 4 from an in vivo experiment shows the parallelism in behavior of two electrodes one in each of the carotid blood-streams. We have observed such reproducibility so often that we feel that our results on the arterial and venous bloods are comparable on a qualitative basis.

Though there is uniformity in behavior of similar electrodes in similar fluids to changes in pH the behavior in dissimilar fluids is variable. This is shown in tables 2 and 3, in which the results of sixteen experiments (using forty-four electrodes) on phosphate mixtures, egg albumen and blood plasmas are given. Column two gives the fluid studied. Column three gives the pH ranges of the fluids used, as determined with the hydrogen and quinhydrone electrodes. Column four gives the pH change, and column five the E.M.F. change in mv. per 0.1 pH. The results are summarized in table 3. The sensitivity of the electrodes varied from 6.19 to 12.62 mv. per 0.1 pH. The lowest sensitivity of the electrode was observed in the phosphate mixtures with an average change in E.M.F. of 8.23 mv. per 0.1 pH, and the highest sensitivity in blood plasma with an average change of 10.87 mv. per 0.1 pH. In egg albumin solutions the electrode showed an intermediate sensitivity of 8.33 mv. per 0.1 pH.

In vivo experiments. Two distinctly different methods were used in the animal experiments. The first method, shown in figure 1, has been abandoned for simpler arrangements, but since it eliminates the employ-

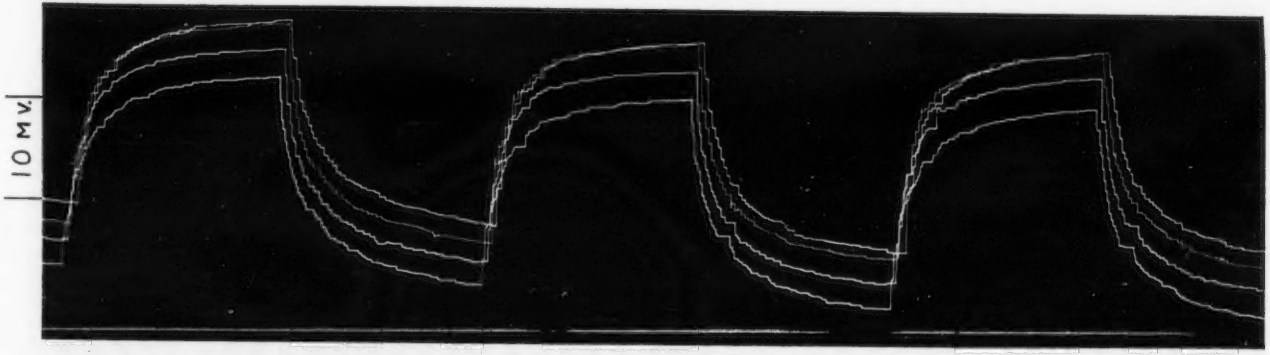

Fig. 3. Smoked record showing changes in acidity synchronously recorded by four manganese dioxide electrodes in the in vitro experiment. Downstroke indicates decreased acidity.

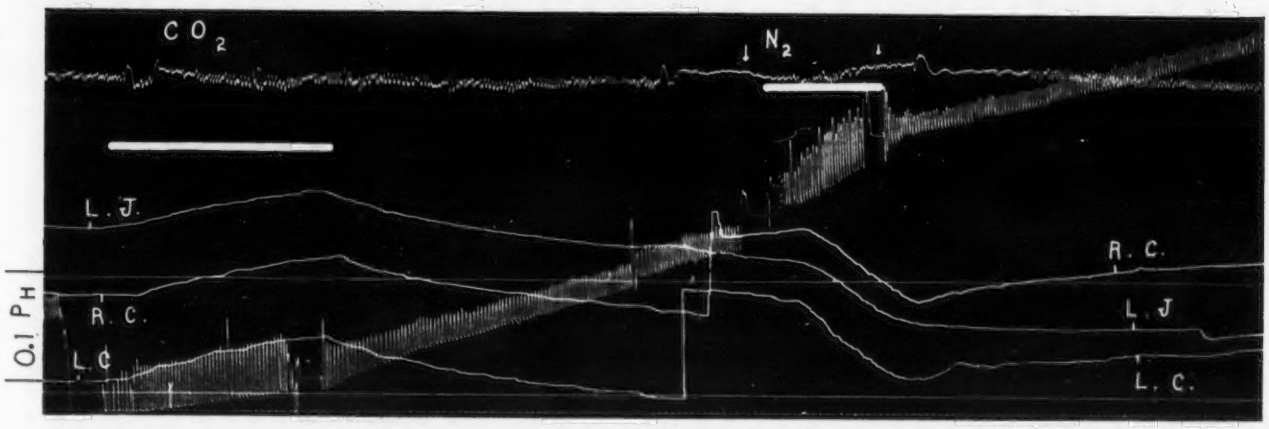

Fig. 4. In vivo experiment showing changes in acidity in the circulating blood of the left and right carotid arteries and left external jugular vein on the administration of carbon dioxide and the intravenous injection of sodium bicarbonate. The similarity of the acidity curves in the carotid arteries demonstrates the reproducibility of behavior of electrodes in the in-vivo experiment.

ment of expensive anticoagulants, and with certain precautions yields reliable data it is briefly described. A specially constructed electrode vessel with a central tube extended in the form of a cannula surrounded by a water jacket served to bring the blood at body temperature to the electrode. With an electrically driven syringe of 100 cc. capacity the blood was drawn from either artery or vein past the electrode at a constant rate of about 100 cc. in forty minutes. Coagulation of the blood was prevented by M/6 sodium oxalate solution delivered at body temperature through a fine capillary tube running through the central tube of the electrode vessel to the very tip of the cannula, insuring a mixture of the blood with the oxalate solution immediately upon leaving its natural vessel. The oxalate solution flowed at about one-tenth the rate of the syringe flow. To prevent unevenness of the oxalate flow due to the oscillations in blood pressure the reservoir of oxalate solution was raised well above the animal (8 meters). Later a small syringe operated in unison with the blood syringe served to supply the oxalate. This arrangement gave smooth records with the arterial blood but the venous records were decidedly irregular. The irregularity proved to be due to uneven mixing of the blood and oxalate solution, and was overcome by an electrically operated pulsator. The pulsator tapping the rubber tube leaving the electrode vessel sent a series of waves into the vein which produced a thorough mixing of the oxalate solution and blood before they reached the electrode. The toxicity of the oxalated blood prevented its reinjection. To delay the harmful effects of loss of blood large dogs were used and the blood replaced with blood substitutes.

It was the inability to obtain hirudin that led to the development of this method, and initial failure with heparin to its continuance. On the venous side the method apparently yielded reliable data under all conditions but on the arterial side changes in blood pressure were accompanied by changes in E.M.F. An increase in blood pressure was associated with an apparent increase in acidity and the reverse held for a fall in blood pressure whether the pressure changed on the administration of CO_2 or mechanical pressure on the abdomen. These effects of blood pressure proved to be due to changes in the proportion of oxalate solution and blood reaching the electrode. The difficulty of correcting this disturbance, which involves the elimination of stretch of the electrode vessel and its connecting tubes, led to another trial of heparin, which proved successful.

The electrode was placed in the natural course of the blood of the heparinized dog. A simple combination electrode and blood sampling cannula of the design shown in figure 2 was inserted, like the ordinary T-shaped cannula, into either the carotid artery or external jugular vein. A calomel electrode was connected with the left tube, the manganese dioxide electrode inserted into the central tube, and blood samples drawn from the right

tube. The blood sampling tube was of barometer bore, provided with a two-way stop cock and sealed with a heavy rubber membrane. By piercing the rubber membrane first, then opening the cock and inserting the syringe needle directly into the blood stream, and similarly withdrawing the needle in two stages blood samples were taken without extra loss of blood. Operative preparations were made with a thermo-cautery knife to minimize the oozing of blood following the administration of heparin. The femoral arteries were used to register mean blood pressure and for hemorrhage, the left femoral vein for intravenous injection, and the trachea for recording respiration and for the administration of gases with the rebreathing device. To insure a free flow of blood past the electrode the electrode vessels were carefully aligned and rigidly fixed in the natural position of the vein and artery. This is particularly necessary for the venous electrode and as a further precaution the opposite external jugular vein was tied to shunt its flow of blood through the remaining patent veins. The electrode vessels were inserted last, followed immediately by the intravenous injection of heparin. The amount injected varied with the strength of the preparation. A few preparations were exceedingly weak. Injection of a gram or more failed to prevent clotting. Fortunately this valuable preparation appears to be improved with the use of newer methods of manufacture. At least the last three lots have been entirely satisfactory. Intravenous injection of 25 to 30 mgm. per kilogram of body weight sufficed to prevent clotting and the collection of fibrin on the electrode for a period of four or five hours. No harmful effects from the administration of heparin have been observed.

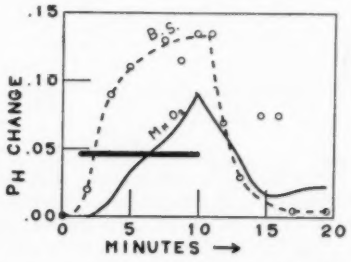

Fig. 5. In-vivo experiment showing effects of rebreathing pure oxygen on arterial pH. A comparison of the manganese dioxide acidity curve with the curve established on individual blood samples by the hydrogen electrode. The period of breathing is indicated by the heavy bar. The pH change is plotted on the ordinates against time in minutes on the abscissas. Oxalate method used.

Though the in vitro experiments indicated the reliability of the electrode for our problem, the final test of the use of the electrode in body fluids of the living animal rests on checks with standard methods of hydrogen ion determinations. These checks have been made both with the hydrogen electrode and with the quinhydrone electrode (9) in a number of procedures employed to study the chemical regulation of respiration.

Early in the animal experiments with the use of the hydrogen electrode we had satisfied ourselves of the value of the manganese dioxide elec-

trode in such procedures as the administration of carbon dioxide, sodium carbonate, ammonium chloride, and mechanical asphyxia. A comparison of the pH curves, obtained with the hydrogen electrode on blood samples

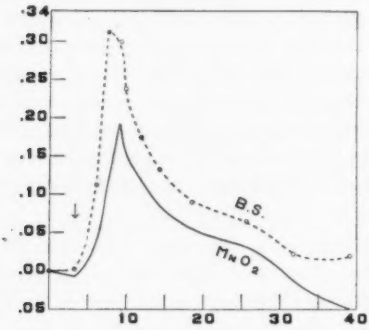

Fig. 6

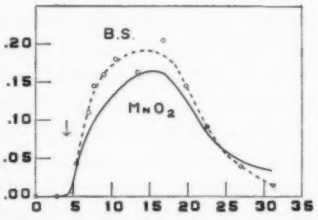

Fig. 7

Fig. 6. In vivo experiment showing effects of rapid injection of lactic acid on arterial pH. A comparison of the manganese dioxide acidity curve with the curve established by the quinhydrone electrode on individual blood samples.

Fig. 7. Changes in arterial pH following intravenous injection of 12 cc. 10 per cent NH_4Cl. Injection at arrow.

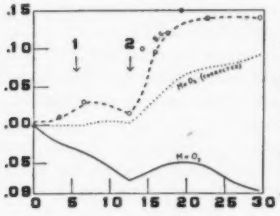

Fig. 8

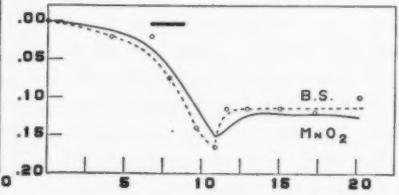

Fig. 9

Fig. 8. Changes in venous pH following hemorrhage and subsequent injection of the blood: hemorrhage at *1*, and injection at *2*. Note the large alkaline drift of the manganese dioxide electrode which is corrected for in the middle dotted record.

Fig. 9. Changes in arterial pH with intravenous injection of 19 cc. $M/2Na_2CO_3$. Heavy bar = injection period.

drawn at regular intervals from the femoral artery, with the continuous curve obtained with the manganese dioxide electrode is shown in figure 5. In transcribing the manganese dioxide acidity curve from the smoked record 10 mv. is taken to represent 0.1 pH. This graph is representative

of the agreement in all our experiments in which the hydrogen electrode was used.

More recently, with the application of the quinhydrone electrode to the determination of the hydrogen ion concentration of blood plasma, we have improved the checks on the behavior of the manganese dioxide electrode

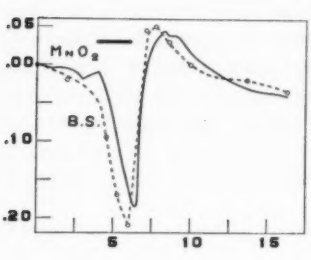

Fig. 10

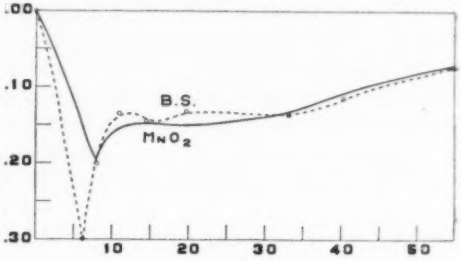

Fig. 11

Fig. 10. Changes in arterial pH with administration of nitrogen. Constant artificial respiration. Heavy bar = period of nitrogen administration.

Fig. 11. Changes in venous pH following intravenous injection of 30 cc. 10 per cent Na_2CO_3. Injection at start of record.

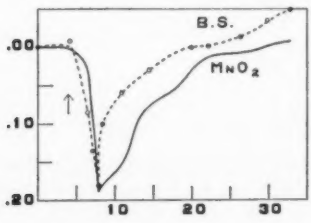

Fig. 12

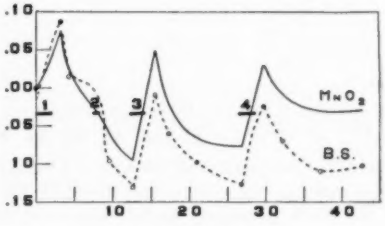

Fig. 13

Fig. 12. Changes in arterial pH following intravenous injection of 8 cc. M/100 NaCN. Injection at arrow.

Fig. 13. Changes in pH of the venous blood with following procedures: at *1*—breathing 20 per cent CO_2; at *2*—injected 200 cc. 5 per cent sodium lactate (neutral); at *3* and *4*—breathing 20 per cent CO_2. Note that MnO_2 values are at a somewhat more acid level at the end of the experiment than the quinhydrone values.

in the circulating blood. The quinhydrone electrode permits several determinations in a relatively short time on small samples of blood. It was, therefore, possible to establish a sufficient number of points on the quinhydrone acidity curve to permit a close comparison with the manganese dioxide acidity curve. All the data represented in the quinhydrone acidity

curves were obtained with the combination electrode and sampling vessel which were aligned to yield samples of blood which had just passed the electrode.[3]

The behavior of the manganese dioxide electrode, as checked with the quinhydrone electrode, is shown in figures 6 to 15, the effects of injection of lactic acid in figure 6, ammonium chloride in figure 7, hemorrhage in figure 8, intravenous injection of sodium carbonate in figures 9 and 10, and the administration of low oxygen in figure 11, mechanical asphyxia after the administration of room air and after pure oxygen in figure 12, the intravenous injection of sodium cyanide in figures 13 and 14, and sodium cyanide followed by sodium lactate and carbon dioxide in figure 15.

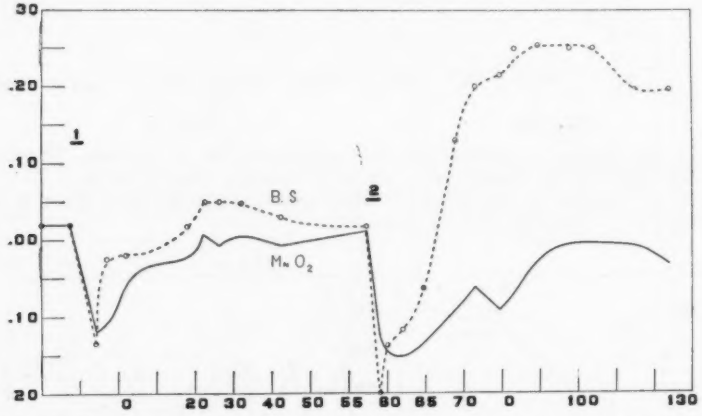

Fig. 14. Changes in pH of arterial blood bollowing intravenous injection of sodium cyanide: at *1*—injected 20 cc. M/100 NaCN; at *2*—injected 33 cc. M/100 NaCN. Note agreement in pH values following first injection; disagreement following second injection.

These graphs were obtained from experiments on eight different animals and with eight different electrodes. In transcribing the manganese dioxide electrode curves 10 mv. is taken again to represent 0.1 pH (the reason for taking 10 mv. is explained below). In those experiments in which drift was very small no correction was made. In those in which drift was appreciable correction was made by determining the acid gradient over a relatively long period in which the condition of the animal was presumably

[3] Clotting of the blood samples, which was likely to occur even though in vivo clotting was absent, interfered with the accuracy of pH determinations. This difficulty was avoided by drawing a drop of heparin solution in the syringe before taking the blood sample. Heating of the platinum electrode in the alcohol flame after each determination seemed to improve the accuracy of the method.

constant. Such corrections are seen in figure 8. It should be pointed out that these corrections are subject to error. In the first place we do not know why the drift varies in different experiments and, therefore, have no means of knowing whether the conditions producing the drift are constant throughout an experiment. On the whole the drift tends to disappear as the experiment proceeds. This is likely to lead to over-correction. The only safe procedure is to check the drift with other electrodes.

Other than this the graphs require little explanation. They present the data in the simplest and clearest form. They show what may be expected from the manganese dioxide electrodes under a variety of conditions. On the whole there was very close agreement in configuration of the curves. The main differences appear in the relative quickness of the electrodes and the apparent difference in sensitivity. Seemingly less

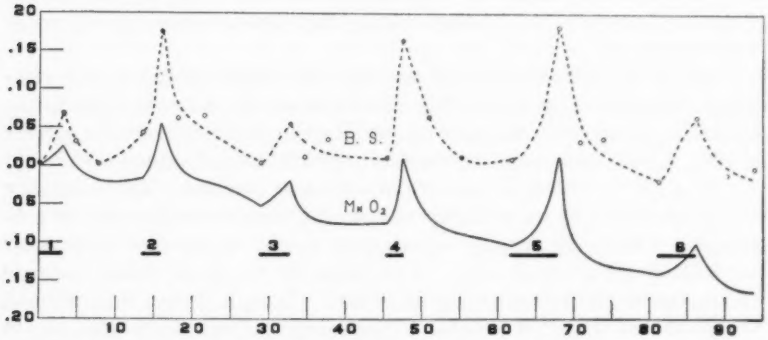

Fig. 15. Changes in pH of arterial blood with mechanical asphyxia. Solid bars = duration of asphyxia: *1*, *3*, *6*—asphyxia with room air: *2*, *4*, *5*—asphyxia with approximately 100 per cent oxygen.

pronounced sensitivity of the manganese dioxide electrode in the in vivo experiments was undoubtedly due to the failure of the electrode to come into complete equilibrium with the blood before a reverse change in acidity occurred. We tried to correct for this factor in transcribing the manganese dioxide acidity curve by taking a lower E.M.F. change per 0.1 pH than the average figures given in table 2. In some of the graphs this correction satisfied the curves completely, in others not quite as perfectly, but only in the observation on sodium cyanide is there a very obvious discrepancy in results. It will be noted in figure 13 that despite the general similarity in configuration of the curves the manganese dioxide electrode was considerably slower. This appears again in the first observation in figure 14. The second observation, on the effects of a considerably larger injection of sodium cyanide, shows a greater discrepancy.

We cannot connect this discrepancy in results with oxygen pressure. We have shown, in agreement with others, that in the in-vitro experiments oxygen exerted no effects on the behavior of the electrode. Alternate phosphate mixtures of the same pH but with 0 and 760 mm. oxygen pressure respectively developed the same E.M.F. The same held for plasma. Similarly in other in vitro experiments we found that the addition of cyanide to either phosphate mixtures or plasmas had no effect on the behavior of the electrode. Neither can the discrepancy of results be related to changes in the velocity flow of blood past the electrode for that factor proved to be of negligible importance. A large reduction in a rapid flow had no significant effect on the E.M.F. developed. A reduction from a relatively slow flow of plasma of 6.5 to 3.0 cc. per minute in the in vitro experiment was accompanied by an apparent increase of acidity of only 0.01 pH, and only when the flow was completely stopped was there a distinct change in E.M.F. in the acid direction amounting to approximately 0.03 or 0.04 pH.

Possibly the delayed response and alkaline values yielded by the manganese dioxide electrode following massive doses of cyanide was due to the formation of metabolites, which on reaching the electrode exerted a specific effect. Lactic acid suggested itself but the injection of sodium lactate did not produce the effects of massive injections of cyanide. The possibility of the liberation of more highly reducing substances might well be considered. Obviously cyanide experiments should be checked with other methods of pH determination. Unfortunately the quinhydrone electrode is also an oxidation-reduction electrode. Though Cullen and Biilman demonstrated that it is possible to determine the initial potential due to the pH in serum our experiments on hemorrhage suggest that it will be a sound precaution to use the hydrogen electrode as the final check in experimental work in which reducing substances may vary as a result of disturbed metabolism. We hope to avoid misinterpretation of extraneous potential changes by observing this precaution and perhaps evaluate the significance of oxidation-reduction phenomena in respiratory control.

SUMMARY AND CONCLUSIONS

The need of a continuous method of recording rapid changes in acidity of the circulating blood and other body fluids for the study of respiratory control and acid-base equilibrium has led to the use of the manganese dioxide electrode.

By means of an electrode cannula the electrode is placed directly in the blood stream of a heparinized dog and connected in the usual way with a type K Leeds and Northrup potentiometer. The galvanometer is maintained in balance and the changes in E.M.F. mechanically recorded on

smoked paper by writing points connected with thread to the fine adjustment of the potentiometer.

Reproducibility of behavior of individual electrodes in dissimilar fluids and blood plasmas from different animals was not attained but a high degree of reproducibility of behavior to changes in acidity in the same sample was the rule. Four electrodes placed in the stream of alternating fluids of different pH values gave mutually superimposable curves.

By establishing the position of the curves with standard methods the manganese dioxide electrode would, therefore, seem to meet the requirements for many biological problems. A comparison of records of acidity changes in the circulating blood with curves established by the discontinuous method of pH determination on individual blood samples offered direct proof of the value of the method in animal experiments. On the administration of carbon dioxide, low oxygen, sodium carbonate, ammonium chloride, lactic acid, small amounts of sodium cyanide, in mechanical asphyxia, and in hemorrhage and injection there was close agreement in the behavior of the manganese dioxide electrode and the hydrogen and quinhydrone electrodes. The only gross exception so far discovered was the discrepancy in results with massive injection of sodium cyanide. It was suggested that this discrepancy was due to the increased liberation of reducing metabolites.

Precaution against the effects of these reducing substances was stressed.

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THE EFFECT OF SOYBEAN FEEDING ON THE BLOOD LIPASE OF RABBITS

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At the present time three kinds of lipase of different origin are known in the animal organism: so-called serum lipase, liver lipase and pancreatic (intestinal) lipase. According to Rona, Pavlovic and Bach (26), (28) these differ in their behavior toward quinin, atoxyl and sodium fluoride, as can be seen from table 1.

Rona and Bach (26) and Rona and Reinicke (29) found that the sensitiveness of blood serum lipase toward quinin and atoxyl varies in different animals.

According to Hess (10), the tissues of the body are a very important source of lipase. There is a certain equilibrium between the tissues and the blood, so far as the lipase is concerned, and the disturbance of this balance on either side is followed by a tendency toward reëstablishment. The kidney lipase is characterized by its relatively high glyceryl triacetate action, low methyl butyrate, high isobutyl acetate, and in general, acetate values nearly the same as, or higher than, corresponding butyrate values; the liver lipase, by high glyceryl triacetate and high methyl butyrate, as well as a general high hydrolyzing action on all the esters; the lung lipase, by a very low glyceryl triacetate action, especially in the more dilute solutions, and butyrate values higher than those of the corresponding acetates (8).

The presence of lipase in numerous foods of vegetable and animal origin led us to suppose their lipase might also be one of the sources of lipase supply in a living organism. Neumeister (23) has suggested that most of the enzymes of the body are absorbed from the intestinal tract. To investigate this question feeding experiments on rabbits were started, using the soybean as the source of lipase.

EXPERIMENTAL. *Method.* Rabbits were fed for intervals of 3 to 9 days exclusively with raw soybeans, autoclaved soybeans or boiled millet and raw cabbage. Blood was taken from the marginal ear vein and tested for lipase at the end of each of these intervals. Rabbits were chosen for this experiment because of their high blood lipase content (24) and because of the uniformity of the figures for blood serum lipase in these animals

during a certain period of time (10). For the determination of lipase Loevenhart's method was used (31). It consists in incubating the mixture of enzyme and zymolyte, i.e. the serum to be tested and ethyl butyrate, in a given dilution at 38°C. for a period of 24 hours, and titrating the acidity developed with 0.1 N NaOH solution using phenolphthalein as indicator. The initial acidity of the ethyl buytrate added is subtracted. The lipolytic power is thus represented by the number of cubic centimeters of 0.1

TABLE 1

Variation in sensitivity of different lipases

LIPASE	DEGREE OF SENSITIVENESS		
	To quinin	To atoxyl	To NaFl
Blood serum lipase	High (the effect is irreversible)	Great (the effect is irreversible)	Great
Liver lipase	None to doses 1000 times higher as compared with serum lipase	Very great (higher than serum lipase) (irreversible)	Great
Pancreatic (intestinal) lipase	Great (the effect is reversible)	None	Slight

TABLE 2

Titration values of the lipolytic activity of 1 cc. serum on ethyl butyrate (cc. of 0.1 N NaOH)

RABBIT	JUNE 2	JUNE 6	JUNE 13	JUNE 30	AUGUST 11	AUGUST 25	SEPTEMBER 8	SEPTEMBER 22
I	0.35	0.41	0.40	0.44				
IV	0.35	0.32	0.30	0.36				
XI					0.32	0.32	0.36	0.36
XII					0.25	0.27	0.32	0.33
XIII					0.44	0.42	0.47	0.43
XIV					0.39	0.37	0.40	0.35

N alkali required to neutralize the fatty acid produced by the enzyme action of 1 cc. blood serum on the ester. It has been found that serum lipase does not deteriorate even when kept for 3 to 4 days in the ice box (3). The determinations were done in duplicate (but without doing blanks), and satisfactory checks were obtained.

Hess (10) found that the lipolytic power of the serum in rabbits remained very uniform from day to day and only occasionally deviated either way. We also investigated this question as a means of control.

The lipolytic activity of the serum of six normal rabbits fed on boiled millet and green vegetables, was tested for periods of 28 and 42 days, using Loevenhart's method, and satisfactorily uniform results were obtained, as can be seen from table 2.

TABLE 3

Resistance of soybean lipase to high temperatures

SOYBEAN PREPARATION	MANIPULATION	ADDED	INITIAL ACIDITY (IN CC. OF 0.1 N NaOH)	FINAL ACIDITY AFTER INCUBATION FOR 24 HOURS AT 37°C. (IN CC. OF 0.1 N NaOH)
Milk, 10 cc.*	Heated for 30 minutes in a boiling water bath	Water, 9 cc.; ethyl butyrate, 1 cc.; toluene, 0.3 cc.	3.8	3.8
Milk, 10 cc.*	Same	Water, 5 cc.; soybean oil emulsion† 5 cc.; toluene, 0.3 cc.	1.9	3.3
Milk, 25 cc.*	Heated until boiling occurred	Water, 100 cc.; ethyl butyrate, 2 cc.; toluene, 2 cc.	8.9	8.9
Milk, 5 cc.*	Same	Water, 4 cc.; ethyl butyrate, 1 cc.; toluene, 0.3 cc.	2.4	2.4
Milk, 5 cc.*	Same	Soybean oil emulsion‡ 5 cc., toluene, 0.3 cc.	1.6	1.6
Milk, 5 cc.*	Autoclaved at 120°C. for 1 hour	Water, 4 cc.; ethyl butyrate, 1 cc.; toluene, 0.3 cc.	2.6	2.6
Milk, 5 cc.*	Same	Soybean oil emulsion‡ 5 cc.; toluene, 0.3 cc.	1.7	1.7
Emulsion, 5 cc.§	Same	Water, 4 cc., ethyl butyrate, 1 cc., toluene, 0.3 cc.	2.1	2.1
Emulsion, 5 cc.§	Same	Soybean oil emulsion† 5 cc.; toluene, 0.3 cc.	0.6	0.6

* Yellow soybeans, 1 part; water, 10 parts.

† Refined soybean oil (salad oil of the Nissin Oil Mill Co., Dairen, with no lipolytic activity), 10 cc.; gum arabic, 5 grams; water to 100 cc.

‡ Refined soybean oil, 10 cc.; autoclaved soybean milk, 90 cc.

§ Autoclaved yellow soybeans, 50 grams; water, 150 cc.; ground in a mortar and passed through a cloth.

The presence of active lipase in the raw soybeans fed to our animals was confirmed by their capacity to germinate and by the lipolytic effect on ethyl butyrate of the emulsion prepared from these beans ("soybean milk").

The resistance of soybean lipase to high temperatures was studied by the use of autoclaved soybeans and heated, boiled and autoclaved soybean milk. The method of testing the lipolytic activity consisted in incubation for 24 hours at 37°C. of the soybean preparation in small stoppered Erlenmeyer flasks with ethyl butyrate or soybean oil emulsion and a little toluene to inhibit the action of bacteria. The final acidity was determined by titration of the diluted specimen with 0.1 N sodium hydroxide, using phenolphthalein, and compared with the initial acidity. Every determination was made in duplicate. The results are summarised in table 3. It will be seen that autoclaving and heating to the boiling point effectively destroy the lipase.

Results. The duration of the experiments was from 21 to 62 days. Of eight experimental animals, the serum lipase in six showed a definite rise when the animals were fed on raw soybeans and a fall when autoclaved beans or other lipase-poor food was given, as is shown in the charts. In two animals, nos. V and VII, there was no definite change.

Rabbit V died spontaneously 36 hours after the last serum-lipase test was made and rabbits II and X were killed. In rabbit V at autopsy a severe hemorrhagic muco-purulent gastritis and a catarrhal enteritis were found. The liver showed parenchymatous swelling. Rabbit II showed white spots in one lobule of the liver and a soft yellowish focus with a few greyish opaque areas in the right kidney-fat. On microscopic examination there were necrotic areas, infiltrated with a few leucocytes in the fatty tissue. In preparations from these necrotic areas no crystals of free fatty acids were seen, but in preparations stained for fat the fat cells were found to be filled with numerous small drops of a fatty substance, occasionally with a diffuse coloration of the cells. These drops are possibly mixed free fatty acids, in an early stage before the unsaturated free fatty acids were removed. In rabbit X at autopsy slight scarring of the kidneys was noticed. The mesenteric blood vessels of the small intestines were distended and the mucous membrane of the small intestines was hyperemic and covered with a thick layer of mucus.

Discussion. The presence in the blood serum of an enzyme capable of hydrolysing monobutyrine was discovered by Hanriot in 1896. Neuberg, Rosenberg and Reicher (20), (21), (22) showed that it has the capacity of splitting off lecithin, and Rona and Michaelis (27) found that it also hydrolyses tributyrin. We may, therefore, conclude that this enzyme is a true lipase. Rona and Michaelis came to the conclusion that we have to consider the presence in the blood of only one lipase, without separating it into a true lipase and an esterase. To quote Hess (10), "There seems to be no doubt that the esterases are true lipases and that their action is quantitatively directly applicable to the hydrolysis of natural fats." In a recent article Rona and Bach (26) always follow the

word "lipase" by the word "esterase" in brackets. These considerations lead us to discuss the lipolytic capacity of the blood and tissues on the basis of our experimental data on the hydrolysis of ethyl butyrate.

The existence of soybean lipase was proved and its properties were studied by Falk (5) and Barton (4). This lipase is soluble in water and hydrolyses ethyl butyrate as well as true fats and oils. Barton concluded that soybeans contain more than one lipase.

Tanaka (30) has shown that the lipase of the castor bean is contained in the resting seed as a zymogen and that acid is necessary to convert the zymogen into an enzyme. According to Barton (4) soy and castor beans contain the same lipase or lipases. In the case of animals fed on dry raw soybeans it would be necessary to postulate the conversion of the zymogen into an enzyme by the hydrochloric acid of the stomach. However, our animals were fed raw soybeans which had been soaked in water for 24 hours, and the beans were eaten at any time within the next 24 hours. There can be, therefore, no doubt that the soybean zymogen was by that time converted into a lipase (by CO_2 and organic acids developed during germination).

The autoclaved beans, previously soaked, which were used as part of the control, had completely lost their lipolytic activity toward ethyl butyrate and soybean oil emulsion (see table 3).

Rabbit VII was the only animal which did not show any response in the serum lipase activity to the ingestion of raw soybeans. It was the only Belgian hare included in the series. Hess (10) found that the concentration of lipase in rabbit's serum was not constant for the species. It may be that this is the explanation of the failure of this animal to respond.

Possibly the general tendency of the blood lipase activity curve in rabbits X, IX, VIII and V, showing a drop toward the end, is partly

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Chart 1. Rabbit II. Titration curve of the lipolytic activity of 1 cc. serum on ethyl butyrate.

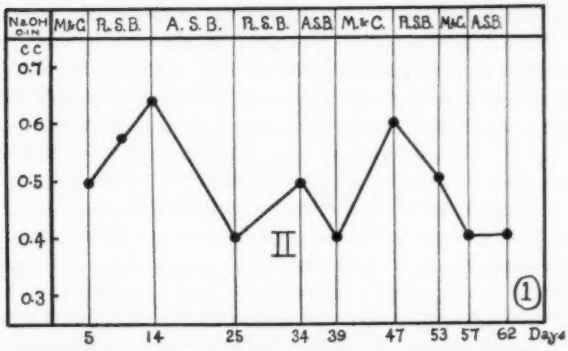
Food: M. & C.—boiled millet and raw cabbage. R.S.B.—raw soybeans, previously soaked in water for 24 hours. A.S.B.—autoclaved soybeans (120°C. for one hour).

Chart 2. Rabbit III. Titration curve of the lipolytic activity of 1 cc. serum on ethyl butyrate.

Food: M. & C.—boiled millet and raw cabbage. R.S.B.—raw soybeans, previously soaked in water for 24 hours. A.S.B.—autoclaved soybeans (120°C. for one hour).

Chart 3. Rabbits VI, VII, VIII, IX, and X. Titration curves of the lipolytic activity of 1 cc. serum on ethyl butyrate.

Food: M. & C.—boiled millet and raw cabbage. R.S.B.—raw soybeans, previously soaked in water for 24 hours. A.S.B.—autoclaved soybeans (120°C. for one hour).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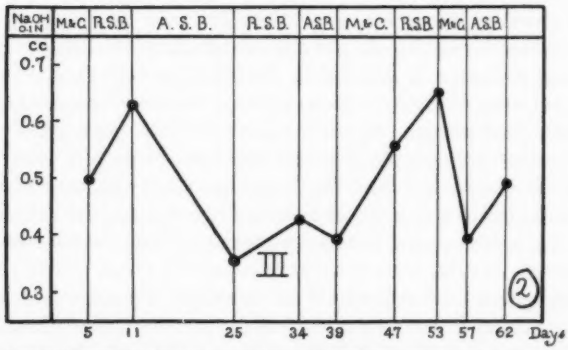
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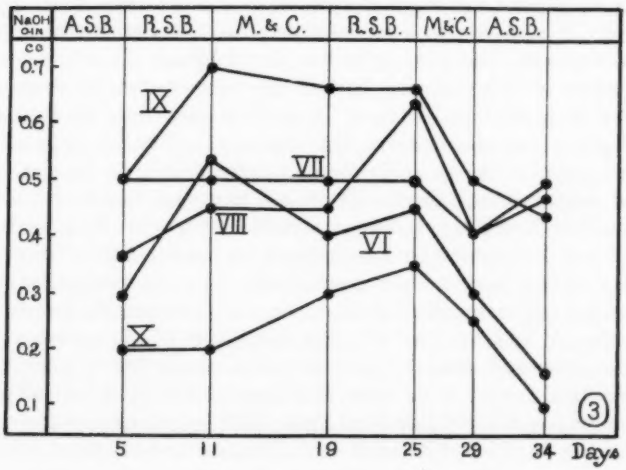
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IX
VII
VIII
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X
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29
34
Days

due to the fact that the diet was restricted to soybeans for a prolonged period (1).

Rabbit V shows a drop in the lipase activity curve while on a diet of autoclaved soybeans. According to Azuma (3) immediately before death from starvation the lipase content of serum decreases abruptly. The last determination of blood lipase in this rabbit was done 36 hours before death occurred, and it can be seen that even then the rabbit had responded to raw soybean ingestion so that no further drop occurred. It was found that in general rabbits eat much more freely of the autoclaved soybeans than of raw soaked soybeans; nevertheless, the quantity of raw soybeans taken was sufficient to produce a rise of the blood lipase. According to Azuma, the blood lipase in starving rabbits shows a rise to 3.5 times the initial figures, but the beginning of the rise was noted by him only on the 14th day of absolute starvation, and 6 hours after a meal was given the lipase reached the normal level. In our experiments we feel that starvation cannot be the cause of the rise in the lipolytic activity.

The high per cent of oil contained in the soybeans (15 to 18 per cent) which our rabbits were fed, may be supposed to act as a physiological stimulus for pancreatic secretion. The lipase of the latter, according to Hiruma (12), is reabsorbed from the intestinal tract and may produce a rise in the blood lipase content. But in our experiments the rabbits were fed periodically with raw and autoclaved beans. The lipolytic activity of the latter is zero, but the percent of fat remains the same. It can be seen from our charts that in the majority of cases the summits of the curves (corresponding to the greater lipolytic activity) were reached after feeding raw soybeans for a few days and the valleys after autoclaved soybeans were fed. It is clear that some additional factor must still be present in the raw soybeans, and this, we believe, is the lipase.

According to Falk (5), very little if any esterase (active toward ethyl butyrate) is present in soybeans, in spite of their high lipolytic power. Our results are not in accord with this conclusion. The rate of inactivation of lipase preparations by acids does not appear to be as great as that of esterase preparations. Barton (4) found that the optimum acidity for castor and soybean lipase is approximately 0.5 per cent (HCl) for lard and olive oil and that neither soy nor castor bean lipase is active toward ethyl butyrate in less than 0.4 per cent acidity. In our experiments, which were carried out on unacidified blood serum, it is clear that the rise in the blood esterase was not due to simple absorption of the soybean esterase from the intestinal canal. The absorbed soybean lipase may undergo some essential change in the liver, as is supposed by Hiruma (12) to occur with the pancreatic and intestinal lipase, and it may acquire the capacity to hydrolyse ethyl butyrate at the pH of normal blood serum. This point of view is supported by the fact that the hydrolysing effect of the soybean

lipase (on lard and olive oil) was shown not only in an acid, but also in a neutral medium. To quote Falk (6): "The hypothesis to be suggested is that the active grouping of the esterase and lipase preparations is of the enol-lactim structure, $-C(OH) = N-$, the specificities being dependent in part upon the groups combined with the carbon and nitrogen and the inactivation consists primarily in a rearrangement to the keto-lactam group $-CO-NH-$" (based on experiments performed on castor bean lipase). It seems, therefore, probable that a foreign lipase, absorbed from the intestinal tract, must undergo changes and be transformed into a lipase specific to the animal, as occurs in the case of proteins and fats.

Another theory might be advanced to explain the effect of the soybean on the lipolytic activity of the blood. Falk (6) showed the production of an ester-hydrolyzing substance by the action of alkali on proteins. The preparations used were obtained from castor beans and consisted of: 1. An albumin-like body more active toward ethyl butyrate than toward glyceryl triacetate. 2. A globulin-like body, more active toward glyceryl triacetate than toward ethyl butyrate. Hulton-Frankel (14) prepared similar substances from soybean globulin and soybean glutelin. Falk and Nelson (7) have shown that amino acids are also capable of hydrolysing esters, and speak of the action as specific. It is possible that some of the products of digestion, as directly absorbed into the blood or as modified by the intestinal wall, augment the lipolytic acitivity of the blood.

From the curves of rabbits II, VIII and X it can be seen that the maximum lipolytic activity on the raw soybean diet was not reached at once but only gradually. This may be explained on the basis of the data of Hess (10) showing that all the tissues of the body take part in the regulation of the blood lipase level. The blood lipase curve will reach its maximum only after the saturation of other tissues with lipase is accomplished.

In rabbit II necrotic foci were found in the kidney-fat. The lipase activity curve of this animal was always high, and possibly this may account for the necrosis. The pericardial and perinephric fat of normal animals has been found to possess a high lipolytic activity, but markedly less than subcutaneous fat (18). This is in accordance with the fact that, during inanition, the fat in the former localities is the last to disappear. In 1889 Fitz called attention to the association of disseminated "fat necroses" with pancreatic disease. Langerhans found that the formation of fat necroses is associated with a splitting of the fat molecules into fatty acids and glycerine. The latter, being soluble, is washed away, whereas the fatty acids are deposited in typical needles, which later, combining with calcium, give irregular, granular masses. Flexner (9) has shown that the necroses contain a ferment capable of splitting butter fat, and that normal adipose tissue contains such a ferment in much smaller amount, if at all.

It seems probable that this ferment is the fat-splitting enzyme of the pancreas, which, as a result of pancreatic disease, has escaped in considerable quantity. Hewlett (11) and recently Hiruma (12) have confirmed this suggestion by direct experiment. According to Hess (10), ligation of all the pancreatic ducts in dogs gives no deviation from the normal lipolytic power of the serum. But Hess only determined the lipolytic activity one week after the ligation was performed and, according to Hiruma, under these circumstances the lipase rises to a maximum on the 4th day and returns to normal on the 8th day.

In 1920 Horvath (13) wrote: "Besides the mentioned diseases (in cattle) white, hard, cheese-like foci were sometimes found in the kidney fat, occasionally involving the whole kidney fat. . . . . Microscopical investigation shows the picture of fat necrosis with the formation of crystals of free fatty acids. The cause is unknown. Special investigation is necessary. It is possible that the disease begins with the white spots which I have observed many times on especially fattened carcasses" (observed in the slaughter house in Tsingtau, China). Similar white spots were sometimes found in the subpleural fat in these cattle. No lesions were found in any other organ. Chemical analysis of the necrotic fat made by Horvath showed free fatty acids (calculated as oleic acid) to be present to the extent of 2.15 per cent; the iodine number was 32.1. The acidity is high and the iodine number low.[1] The data prove that free fatty acids are present in the necrotic fat and that they are mostly saturated acids (stearic and palmitic acid). These cattle were fattened on a diet containing a large amount of soybean cake and black soybeans. According to Lewkowitsch (17) and Falk (5), the soybean lipase is derived from the soybean cake. We have in these pathological cases the results of feeding large amounts of soybean for a long period. It is of interest to note that fat necroses were never observed by Horvath in Mongolian cattle, fattened on grass (examined in Tientsin).

The high figures for serum lipase after raw soybean ingestion must be of importance for the hemolytic and hemo-agglutinating phenomena in the body (20), (21), (22). According to Olsen and Goette (24), there is some parallelism between the lipase and complement content of specific sera. Judging by the findings of Rogers (25), an increase in the blood lipase, following soybean feeding, will probably increase the resistance of the organism for tuberculosis. Horvath's comparative study of the oc-

[1] Lewkowitsch (15) gives the iodine number for the kidney fat of cattle as 40.84 to 29.50. The iodine number for soybean oil, according to the same author (16), is 137 to 143. Kidney fat from cattle fattened on soybean cake always has a low melting point and a comparatively high iodine number. Therefore, an iodine number of 32.1 is abnormally low for such cattle.

currence of tuberculosis in cattle of Central China and Mongolia showed that about 20 per cent of the Mongolian cattle have tuberculous lesions, whereas those of Central China, fed partly on soybean, have practically none. Adolph (2) after a study of the Chinese dietary suggested a possible relation between a diet of bean curd and other soybean products and resistence to infections.

Since soybeans are not eaten raw by human individuals the question of the possibility of raising the serum lipase by a diet containing soybeans still remains open. It must be remembered that in China soybean milk is always boiled for a long time (many hours) in the factories for the purpose of getting the precious pellicula used in the manufacture of other valuable soybean products. Freshly prepared soybean milk heated for 30 minutes in boiling water was found by us to have lost its capacity to hydrolyse ethyl butyrate, but still hydrolysed soybean oil emulsion. Soybean milk boiled for a few minutes at 100°C. did not show any lipolytic activity.[2]

The possibility of the existence of a co-enzyme must be taken into consideration. It was noticed by Magnus (19) that when an extract of liver was subjected to dialysis, the lipolytic power which it originally possessed was gradually lost, but was regained when the dialysate was added. The component which did not dialyse was destroyed by boiling and may therefore be regarded as the enzyme proper, while the dialysable, thermostable body, or bodies, may be called the co-enzyme. If the conditions for soybean lipase are similar, the thermostable fraction present in boiled soybean milk or other boiled products could easily restore the lipolytic activity in the presence of a small amount of some raw food containing the thermolabile part. All raw vegetables might play a part in such restoration. Hence, boiled soybean products as well as raw, under certain conditions may show lipolytic effects. The rise in the serum lipase of dogs in the summer, observed by Hess (10), may possibly be due to the effect of the co-enzyme of some raw food.

SUMMARY

1. The presence of an active lipase in the food tends to cause a rise in the serum lipase activity in rabbits.

2. It seems probable that a foreign lipase, absorbed from the intestinal tract, must undergo changes and be transformed into a lipase specific to the animal, as occurs in the case of proteins and fats.

3. A diet of raw soybeans tends to result in fat necrosis.

[2] In the dry state soybeans withstand a temperature of 100–110°C. for three hours without losing their lipolytic activity (5).

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